

Acta Medica Scandinavica

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EDITORIAL

TERMINAL RENAL FAILURE

For little more than a decade it has been practically possible to substitute or completely replace the kidney in terminal failure by chronic dialysis or by renal transplantation.

The excellent annual registries of the European Dialysis and Transplant Association (EDTA) clearly illustrate the progress during this limited span of time.

The average mortality during the first year of dialysis has decreased from 41% in 1965 to 18% in 1971. In 1971 the average 5-year patient survival on home dialysis, on hospital dialysis, following grafting with kidneys from close relatives and following grafting with necrokidneys, were 77, 52, 65 and 44% respectively.

Between 1963 and 1971 the numbers of centers in Europe (about 430 mill. inhabitants) performing hospital dialysis, home dialysis and renal transplantation have increased from about 40, 5 and 10 to about 480, 115 and 170 respectively. Within the same period the number of patients alive on chronic dialysis has increased from about 200 to about 7 500 and the annual number of kidney transplants from about 100 to about 1 100.

According to most international estimates the number of new patients with terminal renal failure for whom dialysis and transplantation is currently considered indicated is about 40 per million per annum (range 25-50) depending primarily on age and, to a lesser extent, on the nature of the primary renal disease.

The 1971 European record of about 1 100 renal transplants and about 4 000 new patients taken into chronic dialysis thus represents 25-50% of the estimated needs. The average does, however, cover wide variations and a few countries had treatment intensities which were 4 times higher than the European average, indicating that a practical solution to the harassing problem of

providing treatment for all may not be too far off.

Although hardly anybody would contest that a perfectly functioning renal transplant is preferable from all points of view, the present stage of the art is such that transplantation and dialysis are complementary methods of treatment and will in all probability remain so in the foreseeable future. The observed evolution of dialysis clearly indicates that future development must rest upon further extension of self-treatment by home dialysis and upon the design of simpler and still more inexpensive "artificial kidneys".

The most manifest and, hitherto invariably lethal symptoms of terminal renal failure, exudative pericarditis and profuse gastrointestinal hemorrhage, can be completely prevented by proper dialytic treatment. The same applies to uremic polyneuropathy which may become crippling if dialysis is unduly postponed. The pathogenesis of these as well as of other uremic symptoms is obscure, since the uremic toxin(s) remain to be identified. Apart from the production of disposable dialyzers with small extracorporeal blood volume no fundamental progress in the design of "artificial kidneys" has therefore been in evidence.

Studies on the progression and remission of neuropathy before and during dialysis indicate that this lesion is provoked by substances of a higher molecular weight than the conventionally determined indicators of uremic intoxication, urea and creatinine. Work on the *in vitro* toxic effect of readily versus poorly dialyzable fractions of uremic plasma support this contention. Recent studies on dialysis patients, in whom mass removal of small and larger molecules was selectively varied by changes in, *inter alia*, membrane area and dialysate flow in conventional

dialysers, indicate that the uremic toxin(s) are to be found in the range of molecular weight from 500 to 2 000. It remains to be seen whether these experimental approaches will lead to an identification of the uremic toxin(s) and thereby to better possibilities for the design of fundamentally new and simpler types of "artificial kidneys"

Nephrogenic anemia is primarily due to a depression of erythropoiesis. Uremic toxins in all probability play a role but comparative studies on the erythropoietic activity in uremic patients with polycystic kidneys, with contracted kidneys and following total nephrectomy indicate that lack of erythropoietin is also relevant. Biologically active erythropoietin preparations are not yet available. A major practical improvement has, nevertheless, already been made. By minimizing blood loss in the dialyser and by avoiding routine control of blood chemistries, transfusion of blood can be completely omitted in almost all dialysis patients (with the possible exception of those who are totally nephrectomized). Hematocrit values, usually stabilizing around 20 are quite acceptable to most patients. It is relevant to emphasize that the same strict policy with respect to blood transfusions should ideally be observed by departments of general medicine caring for uremic patients in whom dialysis and transplantation may be considered at a later date.

The problem of disturbances in calcium metabolism in terminal renal failure has hitherto only been partly overcome. The discovery of the fundamental role of the kidney for the conversion of natural vitamin D₂ into metabolically active 1,25-dihydroxy vitamin D₃ and the synthesis of this compound, as well as of equally active and cheaper analogues, may however well prove to represent a major step forward in the near future.

The application of chronic dialysis has proven to represent an important tool for elucidation of nephrogenic hypertension. Two types of hypertension can be distinguished. One type apparently the more frequent can be completely controlled through normalization of extracellular volume by dialytic ultrafiltration, supplemented by dietetic sodium restriction. Patients of this type usually have normal levels of plasma renin and angiotensin. Another type can only be controlled by total nephrectomy. Patients of the latter less frequent, type usually have high levels of plasma renin and angiotensin before nephrectomy. Fol-

lowing nephrectomy they may still become hypertensive. If expansion of extracellular volume is not avoided.

The immediate practical importance of these findings is evident, since complete control of high BP can be obtained in most, if not all, hypertensive patients in terminal renal failure without antihypertensive medication which is often difficult to regulate in these cases. Furthermore, the results have shed new light on the pathogenesis of high BP and they have shown that a "renoprival hypertension" does apparently not exist in man. Previous findings of such a condition in animals may possibly be better explained by a species difference in the efficiency of post-nephrectomy care than by a true species difference in the response to the renoprival state.

The totally nephrectomized patient lends himself uniquely to an investigation of the role of the juxta-glomerular apparatus for the regulation of aldosterone secretion. Several studies have already shown that anephric man can increase plasma aldosterone in response to the same stimuli as normal persons, demonstrating that the kidney is at least not the only regulator for the secretion of this hormone. Other new observations of basic physiological importance have been made by comparing the turnover of various plasma proteins in anephric patients to those of normal man thereby shedding additional light on the role of the kidney for their degradation.

As illustrated by the observed constancy of graft survival rate in all large materials, no fundamental progress has been made since whole body irradiation was substituted by cytostatic drugs and prednisone. The recorded improvements in patient survival are almost exclusively due to the fact that an increasing number of patients with graft failure are retransplanted or taken back into chronic dialysis. The most careful studies on antilymphocytic globulin and on lymphocyte depletion (by thoracic duct drainage or blood irradiation) indicate that these methods are of limited effect when added to conventional immunosuppression. Despite their well documented value in transplantation from siblings, current methods for histocompatibility testing are of marginal value if any in necrokidney transplantation. Interest is currently focused on the reactivity of the recipients to a transplanted kidney. Possibly the most immediate advances will come

from studies designed to distinguish "responders" from "non-responders" prior to grafting and from induction of enhancement also in the clinical transplantation situation.

New observations of practical and pathophysiological significance have been made on transplanted patients. One of the most intriguing is the indication that not only patients with renal failure due to genetically determined tubular enzyme deficiency may become improved by transplantation of a normal kidney. Apparently improvement is also observed in patients with renal failure due to generalized enzyme deficiencies such as Fabry's disease. Interesting new possibilities for further understanding of some inborn errors of metabolism may come from trans-

plantation of tissue with adequate concentration of the lacking enzyme.

The days of the pioneers opening up new land for exploitation is always followed by the hard work of cultivating the new soil. In hardly any other field of medicine has the initial enthusiasm of the pioneering work been followed by so many multifaceted problems as within the field of dialysis and renal transplantation.

It has been the purpose of this article to show that, working bedside and benchside, medical science will always overcome its problems and that progress in one field will invariably shed new light also on other fields of clinical and basic science.

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CLINICAL EXPERIENCES WITH PRESERVATION OF NECROKIDNEYS

The Effect of Pretreatment with Chlorpromazine and the Use of a Perfusate which Mimics the Intracellular Ion Composition

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Abstract Sixty-one necrokidneys have been preserved by means of hypothermic storage following short initial cooling perfusion with perfusate which mimics the intracellular ion composition. Five kidneys failed to function due to acute vascular rejection, but the remaining 56 functioned satisfactorily at some time after transplantation, the criterion being that the lowest obtained serum creatinine in the recipient reached normal or nearly normal values. Fifty-seven kidneys originated from neurosurgical donors, in whom assisted respiration was stopped after demonstration of brain death and from whom the kidneys were removed after cardiac arrest. Four kidneys originated from donors with sudden cardiac arrest. The kidneys from the neurosurgical donors were divided into two groups according to pretreatment of the donor with chlorpromazine. In group 1 (26 kidneys) no chlorpromazine was given and in group 2 (31 kidneys) chlorpromazine 4 mg/kg b.wt. was given just before stopping the assisted ventilation. The pretreatment with chlorpromazine increased the number of immediately functioning kidneys after transplantation from 54 to 85% and, since the two groups were almost similar with respect to factors which may influence the postoperative kidney function, it is concluded that the difference derives from the use of chlorpromazine. No difference in the best achieved kidney function could be demonstrated between the two groups. Six kidneys had cold ischaemic time between 14 and 1 hours (group 3). All these kidneys functioned after transplantation, and normal or nearly normal serum creatinine values were obtained in the recipients.

In earlier papers we have presented our results concerning 24-hour preservation of pig kidneys by means of hypothermic storage following a short initial cooling perfusion with a perfusate which mimics the intracellular ion composition (9, 10, 11). With a warm ischaemic period of 1-3 min consistently good results were obtained in a consecutive series (11). When the warm ischaemic period was extended to 1 hour however con-

sistently good results were only obtained provided that the animals had been pretreated with chlorpromazine (9, 10).

Since April 1970 we have preserved human necrokidneys with this perfusate and since Feb. 1971 we have pretreated the donors with chlorpromazine whenever possible. It is the aim of this study to elucidate whether 1) the use of this perfusate gives consistently satisfactory clinical results, 2) pretreatment of the donor with chlorpromazine has improved the results and 3) the permissible length of the cold ischaemic period (i.e. the period from the start of the cooling perfusion until recirculation in the recipient) in clinical practice is of the same order of magnitude as that found in the experimental work.

MATERIAL AND METHODS

The material includes 61 transplantations with human necrokidneys during the period April 13, 1970-March 13, 1972.

Fifty-seven kidneys came from donors from the Neurosurgical Department. In all these donors brain death had been demonstrated by means of clinical examination, EEG and carotid angiography (5). Following this the artificial respiration of the donors was stopped and, after cardiac arrest, the kidneys were immediately removed. The time from stopping the artificial respiration until cardiac arrest is hereafter referred to as the agonal phase, and the time from cardiac arrest until the start of the cooling perfusion as the warm ischaemic period. Falling BP before the cessation of the artificial inspiration was treated with vasopressor agent (Arminin®). This was necessary in 85% of the cases.

Four kidneys came from patients who developed sudden irreversible cardiac arrest without possibility of resuscitation. In these cases external cardiac massage and

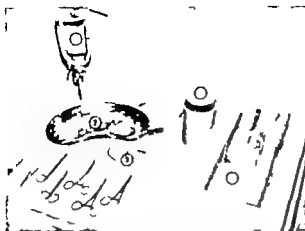


Fig 1 The perfusion and cooling equipment used. (1) Perfusate medium. (2) Bowl with glucose ice in which the kidney is placed during the perfusion. (3) Silicone catheters. (4) Organ container. (A double container the innermost containing the kidney and the outer preserving the sterility of the innermost and ensuring against sudden changes in temperature.) (5) Cooling box for transportation. (Opened to visualize the organ container and cooling elements, Nucleon® A/S Nunc.)

artificial respiration were continued until the start of removal of the kidney. In these 4 cases the terms agonal phase and warm ischaemic period have not been used, since the circumstances were different from those of the other 57 kidneys. The period from onset of cardiac arrest until the start of the cooling perfusion is given.

In 59 cases normal serum creatinine values, normal urinary sediment as well as a negative clinical history with respect to kidney disease, systemic and malignant disorders (except primary brain tumours), diabetes mellitus and hypertension were found in the donors before removal of the kidneys. The remaining kidneys from donors with sudden cardiac arrest were judged normal by macroscopical examination after removal.

In all 61 cases 15 000 IU heparin and 500 ml mannitol 10% were given intravenously. In the 57 donors from the Neurosurgical Department the pretreatment was started after brain death had been diagnosed, just before termination of the artificial respiration, and in the remaining 4 donors after irreversible cardiac arrest. In the period Feb. 13 1971–March 13 1972, 36 kidneys came from donors who had also been pretreated with chlor-

promazine (4 mg/kg b.wt.) administered intravenously in 400–1 000 ml saline or 500 ml mannitol 10%.

The kidneys were removed through a transverse abdominal incision above the umbilicus. The peritoneal sac covering the kidney was incised and kidney renal vessels and ureter dissected as carefully as possible. The renal vessels were then clamped and cut as medially as possible. The ureter was cut about 15 cm from the pelvis.

As soon as possible after removal the kidneys were placed in crushed glucose ice (isotonic glucose) and perfused with 500 ml cooled perfusate (3°C), using a pressure between 100 and 150 cm H₂O. The perfusate is nearly similar to that described as the C₁ solution by Collins et al. (4) using basic solution (containing K₂HPO₄·3H₂O 9.7 g, K₂H₂PO₄ 2.05 g, KCl, 1.3 g, NaHCO₃ 0.84 g, and aqua sterilis ad 1 000 ml) to which papaverine (50 mg/l), glucose (20 g/l) and MgSO (7.38 g/l) were added immediately before use. After this the kidneys were stored at 0–2°C in a solution similar to the perfusate medium, the only exception being that magnesium sulphate was omitted to prevent precipitation of relatively insoluble magnesium phosphates in the storage medium. Fig. 1 shows the perfusion and storage equipment used.

The kidneys were sent to 15 transplantation centres in 7 European countries. The distribution was organized by the Tissue Typing Laboratory in order to get the best possible match between donor and recipient. Histocompatibility as well as the occurrence of leucocyte antibodies in the recipients at the time of transplantation were noted. The degree of histocompatibility was classified according to Kleinmeyer-Nielsen et al. (7) in A, B, C and D matches. Later a questionnaire was sent to the transplantation centres to get information concerning the cold ischaemic period (time from start of the cooling perfusion until recirculation), complications during surgery and in the first postoperative period, rejection episodes and postoperative kidney function (judged by 1) day of onset¹ i.e. the first day on which the transplanted kidney is able to lower serum creatinine and 2) the lowest serum creatinine achieved.

In order to answer the three questions mentioned in the introduction, the material will be described in three groups. Group 1 26 donor kidneys from the Neurosurgical Department not pretreated with chlorpromazine. Group 2 31 donor kidneys from the Neurosurgical Department pretreated with chlorpromazine. Group 3 6 donor kidneys with cold ischaemia of more than 14 hours (4 from donors dying in sudden cardiac arrest and 2 from donors included in group 2).

Table 1. Results of transplantation with necrokidneys in groups 1 and 2, judged by "day of onset" and the lowest serum creatinine obtained in the recipient

	Onset of function (no. of kidneys)			Lowest serum creatinine	
	Immediate	Delayed	Never	(mg %)	(S.D.)
Group 1 (n=26)	14	11	1	1.5 (0.7–2.4)	0.6
Group 2 (n=31)	23	4	4	1.2 (0.6–2.1)	0.4
	37	15	5		

RESULTS

Groups 1 and 2

Table I shows the results of transplantation in the two groups as judged by the "day of onset" and the lowest serum creatinine achieved. Groups 1 and 2 contain one and four kidneys, respectively which never functioned at any time, the reason in all cases being described as acute vascular rejection. The diagnosis was based on histological examination after graftectomy which was done 4, 4, 5, 23 and 30 days after transplantation, and in all cases severe acute vascular rejection was demonstrated. Two of the kidneys (group 2) started just after recirculation with copious urine production, but stopped functioning after a few hours. In group 1 56% of the remaining 25 kidneys and in group 2 85% of the remaining 27 kidneys functioned immediately after transplantation. This difference is statistically significant ($p < 0.05$ Fisher's exact test). The distribution of "day of onset" in the two groups is shown in Fig. 2. It can be seen that "day of onset" in kidneys with delayed onset of function varied between 3 and 64 days (mean 14) in group 1 and between 7 and 13 days (mean 10) in group 2. No statistical difference between the means could be demonstrated ($p > 0.10$ Mann-Whitney rank sum test). A comparison between the lowest achieved serum creatinine in the two groups showed no statistical difference ($0.05 < p < 0.10$ Mann-Whitney rank sum test) (Table I).

Table II. Results of transplantation with necrokidneys in group 1

- number of kidneys					
Warm ischaemia duration (min)	N function (%)		Onset of function		Lowest serum creatinine (mg %)
			Day		
0-10	4	0	Imm. 3	0	1.7 (1.3-2.4)
			Late 1	6	2.0
10-20	12	1	Imm. 6	0	1.6 (1.3-2.3)
			Late 5	3-64	1.3 (0.7-1.8)
20-30	5	0	Imm. 3	0	1.1 (1.0-1.2)
			Late 2	13-15	1.6 (1.3-2.0)
30-41	5	0	Imm. 2	0	1.4 (1.1-1.7)
			Late 3	4-9	1.6 (1.1-1.7)
Total	26	1	Imm. 14	0	1.6 (1.0-2.4)
			Late 11	3-64	1.5 (0.7-2.0)

In Tables II and III the results of groups 1 and 2 are presented according to the length of the warm ischaemic period. No significant correlation between the warm ischaemia duration and non functioning kidneys could be demonstrated ($p > 0.10$ rank correlation test). No significant correlation between the warm ischaemia duration and immediate or late onset of function could be demonstrated either in group 1 ($p > 0.10$) or 2 ($p > 0.10$) using the rank correlation test. Only if the data in the two groups were added was a significant correlation found, i.e. the chance of im-

Table III. Results of transplantation with necrokidneys in group 2

number of kidneys					
Warm ischaemia duration (min)		No function (n)	Onset of function		Lowest serum creatinine (mg %)
				Day	
0-10	10	0	Imm. 9	0	1.1 (0.6-1.5)
			Late 1	9	1.0
10-20	12	2	Imm. 10	0	1.2 (0.6-2.1)
			Late 0	—	—
20-30	6	1	Imm. 3	0	1.3 (0.9-1.7)
			Late 2	7-13	1.0 (0.9-1.0)
30-40	3	1	Imm. 1	0	2.3
			Late 1	10	1.1
Total	31	4	Imm. 23	0	1.3 (0.6-2.3)
			Late 4	7-13	1.0 (0.9-1.1)

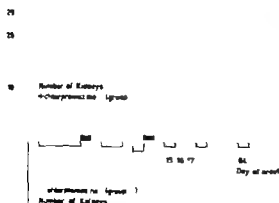


Fig. 2 The relation between day of onset of the kidneys in groups 1 and 2. Kidneys which never functioned have not been noted.

Table IV. Comparison of the agonal phase and the warm and cold ischaemic period between groups 1 and 2

	Agonal phase (min)	(S.D.)	Warm ischaemia (min)	(S.D.)	Cold ischaemia (min)	(S.D.)
Group 1	18 (9-30)	6	19 (5-38)	9	348 (179-795)	205
Group 2	16 (6-23)	6	17 (6-37)	9	374 (103-856)	172

Table V. Distribution of matches in groups 1 and 2

Match	Onset of function (no. of kidney)					
	Group 1			Group 2		
	Immediate	Delayed	Never	Immediate	Delayed	Never
A ₀	2	1	0	1	0	0
B (C)	1	0	0	0	0	0
B (D)	1	3	0	0	0	0
C	6	—	0	11	2	1
C (D)	3	4	1	8	2	2
D	1	1	0	3	0	1

mediate onset of function decreased with the length of the warm ischaemia ($p < 0.05$ rank correlation test). It should be noted, however, that 19 of 20 kidneys in group 2 with a warm ischaemic period of 20 min or less functioned immediately after transplantation irrespective of the length of the cold ischaemic period (13/4-14 1/4 h) or the agonal phase (6-5 min).

In group 1 the kidney which never functioned had an agonal phase of 14 min and a cold ischaemic period of 7 h 25 min. The kidneys with delayed onset of function had a mean agonal phase duration of 15 min (9-23) and a mean cold ischaemic period of 6 h 28 min (2.59-13.15). These values are not significantly different from

the corresponding values for the whole of group 1 as seen in Table IV ($p > 0.10$ Spearman).

In group 2 the kidneys which never functioned had a mean agonal phase duration of 14 min (6-22) and a mean cold ischaemic period of 7 h 25 min (4.30-10.12). The kidneys with delayed onset of function had a mean agonal phase duration of 14 min (10-21) and a mean cold ischaemic period of 6 h 55 min (5.18-10.0). These values are not significantly different from the corresponding average times for the whole of group 2 as seen in Table IV ($p > 0.10$ Spearman).

It can also be seen from Tables II and III that the best achieved kidney function as judged by serum creatinine was not correlated to "day of onset" or the length of the warm ischaemic period, the p -values in all cases being higher than 0.10 (Spearman).

In Table IV the average durations of the agonal phase and the warm and cold ischaemic periods are compared in the two groups. It can be seen that there is no significant difference ($p > 0.10$ Spearman).

The average age of the donors in group 1 was 36 years (17-59) and in group 2 29 years (7-56). No significant difference could be demonstrated ($0.05 < p < 0.10$ t -test).

The distribution of matches in the two groups is shown in Table V. It can be seen that there is a preponderance of poor matches in group 2, but no significant difference could be demonstrated ($p > 0.10$ χ^2 -test with Yates correction). The distribution of matches in relation to "day of onset" is also shown. Four of five non-functioning kidneys were C (D) or D matches. No relation between match grade and "day of onset" could be demonstrated ($p > 0.10$, χ^2 -test with Yates correction).

All recipients were regularly tested for leuco-

Table VI. Distribution of leucocyte antibodies in groups 1 and 2

— number of recipients, with, — without leucocyte antibodies

Group	Onset of function									
	Total		Immediate			Delayed			Never	
	—	—	—	—	—	—	—	—	—	—
1 (n=29)	11	15	14	7	7	11	3	8	1	0
2 (n=31)	10	21	23	5	18	4	3	1	4	2

Table VII *Distribution of age, sex and diagnoses among the recipients in groups 1 and 2*

Group	Age (yr)	Sex		Diagnoses				
		♂	♀	Chr glomerulonephr	Chr pyelonephr	Cong cyst. kidney	Malignant neoplasm	None
1	43 (24-60)	13	11	8	11	3	0	3
2	41 (21-59)	21	10	14	6	3	2	6

cyte antibodies. In group 1 there were 11 out of 26 recipients with leucocyte antibodies in the blood at the time of transplantation and, in group 2, 10 out of 31 recipients. Table VI shows the distribution of leucocyte antibodies in relation to "day of onset". No statistical difference could be demonstrated ($p > 0.10$, χ^2 -test with Yates correction).

Data concerning the recipients are shown in Table VII. It can be seen that chronic glomerulonephritis was the dominating diagnosis in group 2 and chronic pyelonephritis in group 1. No significant difference in age could be found ($p > 0.10$, t -test).

The 11 cases in group 1 with delayed onset of function include two cases with recipient hypotension during the transplantation and one with possible severe rejection already from the second day after transplantation. The four cases in group 2 with delayed onset of function include one patient with a prolonged period of hypotension during and for several hours after transplantation and one with protracted suturing on account of multiple renal arteries. The occurrence of hypotension during transplantation did not induce us to exclude these cases from the material, but the possibility of development of acute tubular necrosis in these kidneys due to recipient hypotension should be kept in mind.

The time for suturing the vessels was only available in 21 cases. The mean time was 41 min (13-60) in 14 cases of group 1 which includes 8 cases of delayed kidney function with an average time of 44 min (28-60). In group 2 an average time of 35 min (26-43) was found in 7 cases. In 6 cases on which information was available concerning kidney surface temperature, this was found to be 8°C in a cage (5-10). The time for suturing the blood vessels in this paper included in the cold ischaemic period.

Group 3

Table VIII shows the results of transplantation using kidneys which had a cold ischaemic period between 14 and 21 hours. In this group are included two neurosurgical donors and four donors with sudden cardiac arrest followed by external cardiac massage and ventilation until the start of the removal of the kidneys. It can be seen that all the kidneys began to function within a reasonable time and that the serum creatinine in five of six cases fell to normal or nearly normal values.

DISCUSSION

One of the objects of any preserving method used for human necrokidneys should be that it gives consistently good results even with kidneys which are already damaged in the agonal phase or during the warm ischaemic period.

It is possible that some preservation methods accelerate already existing tissue damage to a greater extent than others, and it is therefore possible that a method which gives a good result with undamaged kidneys can be directly injurious

Table VIII *Results of transplantation using necrokidneys with a cold ischaemic time between 14 and 21 hours*

Time from cardiac arrest to start of cooling perfusion	Cold ischaemia (h)	Day of onset	Lowest serum creatinine (mg %)	Chlorpromazine
6	14.06	0	1.2	
11	14.56	0	1.6	
42	15.05	10	1.3	
45	21.00	12	1.3	-
46	14.10	22	1.0	-
127	18.00	18	1.1	

to previously damaged kidneys. For example Belzer et al. (2) found that 7 (22%) kidneys out of 32 transplanted human necrokidneys preserved by continuous plasma perfusion never functioned (3 cases with acute rejection not included). No certain explanation was found apart from kidney damage before or during the preservation. In these 7 cases the warm ischaemic period was 15–30 min and the time in the perfusion apparatus only 5–8 1/2 hours. In contrast, other kidneys in this series functioned well after 31 hours in the perfusion apparatus. These authors recognized that the difference between success and failure after transplantation could be correlated with the vascular resistance of the kidneys to perfusion. The results with this preservation method improved markedly after vasospasm had been at least partially eliminated by pretreatment of the donors with phenoxybenzamine (1).

In our material 26 kidneys with an agonal phase from 9 to 30 min, a warm ischaemic time from 5 to 38 min, a cold ischaemic time from 3 to 13 1/4 hours and without pretreatment with vasodilators, all functioned with one exception (acute rejection) at some time after transplantation. This is in accordance with the findings of other workers using the same preservation method (5–12). Since nearly all the transplanted kidneys functioned satisfactorily at some time, the criterion being that the lowest obtained serum creatinine reached normal or nearly normal the preservation method must be judged to be reliable without dangerous side-effects to kidneys.

With respect to the desirability of immediate kidney function after transplantation, only 56% functioned immediately in group 1 the rest starting between 3 to 64 days afterwards. It was not possible to find a correlation between the day of onset and the length of the cold and warm ischaemic periods. This supports the hypothesis that other factors than anoxia affect this relationship. Pretreatment of the donors with chlorpromazine increased the number of immediately functioning kidneys to 85% (group 2), which is a significant increase. No difference in the best achieved kidney function could be demonstrated between the two groups.

It has been shown in experiments with pigs and rabbits that the vascular resistance in kidneys increases during the agonal phase (2) as well as

during the warm ischaemic period (3) and that this increase can be reduced after pretreatment with vasodilators such as phenoxybenzamine (2) and chlorpromazine (3). The results of this work are compatible with the hypothesis that chlorpromazine prevents severe and protracted vasospasm, which retards the day of onset but does not influence the final kidney function.

Evaluation of single factors by intercomparison of clinical preservation results is difficult because of the multiple factors involved, as for example the length of the agonal phase, the warm and cold ischaemic periods, the degree of histocompatibility recipient leucocyte antibodies, surgical technique and the character of the donor and recipient material, which affect or may affect the results. In this work we have judged these factors to be sufficiently similar in groups 1 and 2 to allow the conclusion that the difference in the day of onset between the two groups derives from the use of chlorpromazine. It should be noted, however that this is an uncontrolled trial, as patients were not allocated randomly to either chlorpromazine or controls, and therefore it might be postulated that the difference is a simple consequence of e.g. general technical advances. Previous experiments in pigs (9–10), however, indicate that this is an unlikely explanation.

The results in group 3 show that, even with up to 21 hours cold ischaemia and a relatively long interval from cardiac arrest to the initiation of the cold perfusion, it was possible to obtain a normal recipient serum creatinine. The clinical results therefore seem to endorse the animal experiments in pigs, where one hour of warm ischaemia followed by 24 hours cold ischaemia did not cause irreversible kidney damage if pretreatment with chlorpromazine was used (9–10).

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THE INFLUENCE OF PREOPERATIVE PENSION ON VOCATIONAL REHABILITATION FOLLOWING RENAL TRANSPLANTATION

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Abstract. The vocational rehabilitation and the influence of sick-leave and disability pension has been studied in 88 recipients of primary renal grafts. These 88 were left for study after exclusion from 150 consecutive recipients of renal grafts operated upon between 1965 and 1969 in Göteborg. Of the 88 patients 64 were living, 14 functioning grafts during the postoperative months 20-25. Of the 52 non-pensioned patients in this period 43 were rehabilitated occasionally and 9 were still on sick-leave. Eight of these 9 had returned to employment within the next 12 months. The vocational rehabilitation of the preoperatively pensioned patients in this study was non-existent. Of 12 preoperatively pensioned patients 9 had functioning grafts in the postoperative months 20-25 and had high degree of medical rehabilitation. Preventive rehabilitation to avoid this discrepancy between medical and vocational rehabilitation should start in the pretransplant state, continue during posttransplant dialysis and result in high degree of medical and vocational rehabilitation after successful transplantation.

Reports on vocational rehabilitation following renal transplantation are few (2, 3). In a previous paper we reported on the estimated medical and vocational rehabilitation in 150 recipients of renal grafts observed over a period of 12-60 months (1). The present paper is an extension of that report, dealing particularly with the influence of full pension, given to recipients before transplantation, on the vocational rehabilitation following transplantation.

MATERIAL

One hundred and fifty recipients of primary grafts transplanted Jan. 1965-Oct. 1969: 87 males and 63 females, were studied. Their mean age was 38 years (range 10-61). Diagnoses, pre- and postoperative treatment have been given in previous publications (1-3). Included are both recipients of living donor grafts and of cadaveric kidneys. Graft function more than 4 months as needed for re-

gistration of the patient in this study. Excluded from the study were patients younger than 16 years, and recipients pursuing studies. Since they were not insured, no registration of their sick-leaves existed. One patient had disability pension because of congenital anomalies and was excluded. Remaining for study were 29 recipients of living donor grafts and 59 recipients of cadaveric grafts. The patients with pension were recorded as having 100% sick-leave in the registration of sick-leave.

METHODS

Information concerning sick-leave and pension covering the time until Jan. 1972 was obtained from the local insurance offices of the patients. An estimation of the degree of medical rehabilitation according to the Hansen Kidney Transplant Registry was made by either of the authors by means of personal interview and examination. In a few cases of patients not near this hospital we had to rely on personal interviews by telephone and reports from their local nephrologists.

The postoperative months 20-25 were chosen as representative for the vocational situation of surviving recipients with functioning primary grafts. It was considered that preoperative complications of the uremic and dialytic state (osteopathy, neuropathy) of the patients, as well as complications of the immediate postoperative course (urinary leakage, acute rejection, infections, etc.) should have been dealt with definitely or entered reasonably steady state.

RESULTS

Of the original 88 recipients included in the study 64 were living with functioning grafts during the postoperative months 20-25. These 64 patients used 43% of this period as sick-leave. The 38 recipients of cadaveric grafts used 41% of the total time in the postoperative months 20-25 while the 26 recipients of organs from living donors used 48% in the same period as sick-

leave. The mean age was 40.8 years in the former group of patients and 33.7 years in the latter.

Of 88 patients included in the study 9 had received full pension before the transplantation and another 3 received their pension within 6 months postoperatively based upon their pre-transplant uremic status. These 12 patients are designated as having preoperative pension. Four of them received their grafts from a living donor. In the postoperative months 20-25 the number of patients with preoperative pension had decreased to 9. Two patients had been retransplanted and one had died 8 months after the transplantation. The mean age of the 12 patients with preoperative pension was 43.6 years at the time of operation. The preuremic vocational status of the patients was divided into manual labour, intellectual or clerical work and housework. The distribution of manual labour among the 12 patients with preoperative pension and among the 52 patients in the postoperative months 20-25 without preoperative pension was 75% (9 patients) and 63% (33 patients), respectively. No major differences of occupational distribution existed between the two groups.

A comparison of the pre and postoperative vocational rehabilitation of the 52 patients, non-pensioned in the postoperative months 20-25 showed that 60% had returned to employment similar to that before transplantation. Of the 22 recipients of living donor grafts 46% had returned to employment similar to that before operation. Of the 30 recipients of cadaveric kidneys 70% had returned to employment similar to that before transplantation. Ten of the 52 patients had returned to lighter work, 2 to heavier work. Altogether 9 patients were still on sick leave in the postoperative months 20-25. Within the next 6-12 months 8 of these patients returned to the labour market. One patient received full pension. The distribution of manual labour had changed from 63% preoperatively to 37% among the 52 patients in the postoperative months 20-25. The percentage intellectual or clerical work had increased from 23 to 33%.

None of the patients with preoperative pension had returned to any work in the postoperative months 20-25. The pessimistic situation as regards vocational rehabilitation of the 9 preoperatively pensioned recipients was not easily explained by low degrees of medical rehabilitation. All 9 pa-

tients were classified as belonging to the two highest degrees of rehabilitation, i.e. completely normal (5 patients) and not hospitalized activities moderately restricted (4 patients), according to the 4-grade scale of the Human Kidney Transplant Registry.

A difference was seen in preoperative time on sick leave and pension for two groups of patients surviving with primary grafts in the postoperative months 20-25. The length of the last continuous preoperative time on sick-leave and pension was longer for the 9 patients who received pension preoperatively than for the 12 still on sick-leave or having received pension postoperatively. Eight of the former 9 patients had preoperatively been on sick-leave or pension for more than 12 months and 5 of them for more than 24 months. All 12 patients who received pension postoperatively or were still on sick-leave in the postoperative months 20-25 had preoperatively been on sick-leave less than 12 months. The mean age was 42.4 years (range 23-53) for the 9 patients with preoperative pension but 35.8 years (range 21-57) for the other 12 patients. Pyelonephritis as the cause of uremia was more common (5/9) in the former group of patients than in the latter (3/12). A dominance of women was found among the preoperatively pensioned patients (6/9).

DISCUSSION

In addition to being a life-saving procedure, human renal transplantation aims at rehabilitating subjects, so that they return to the situation they had before entering into chronic uremia. In recipients with well tolerated grafts this is most often the case. In a minor proportion of the patients, due to sequelae from previous uremic, dialytic or postoperative complications, a lower degree of rehabilitation remains indefinitely. Some of the most common and disabling conditions in this group of patients are peripheral neuropathy, generalized osteopathy or avascular necrosis of the femoral head.

The present report draws attention to the discrepancy between the low degree of vocational rehabilitation despite a high degree of medical rehabilitation in a series of preoperatively pensioned subjects. Two main questions arise regarding this group. What were the circumstances in connection with their obtaining full pension be-

fore transplantation? Why were pensions not annulled following successful transplantation in recipients with high degrees of medical rehabilitation?

As regards the first question there are three elements involved in the decision. The patient makes a request, the doctor issues a certificate based on his examination and knowledge of the patient, and the federal pension insurance council ultimately reaches the decision. In this sequence of events the patient must be regarded as the least responsible part. Usually he has experienced a progressively diminishing working capacity for a long period which he relates to his renal disease. More or less reluctantly he has accepted his state of being unemployed and has adapted to it. It is likely that this group of patients does not include those who, sometimes aggressively demand to be cured by their doctors. In such a situation they could hardly escape from being informed of the possibility of dialysis and renal transplantation.

The role of the pension insurance council is also quite understandable. The council is informed of a chronic, serious illness in the patient, who has been unable to attend his regular job for a considerable period. It is beyond their duty to judge whether active treatment should be advocated in that particular case or not. They might ask the certifying doctor for a supplementary statement if chronic dialysis or renal transplantation had been considered.

The doctor who issued the certificate should be the one who at this stage withholds premature pensioning of the patient. By tradition a pessimistic opinion as regards the future life and working capabilities of patients with chronic uremia has been held by many doctors. However medical progress in the last decade with improving experience of chronic dialysis and renal transplantation, has rendered such a defeatist attitude to the uremic patient ungrounded. Naturally a time lag must exist before the information on new medical methods of treatment reaches the general practitioner. Furthermore the resources for active treatment of chronic uremia are insufficient to take care of all suitable uremic patients. The available resources have increased in this country but not as fast as the request for active treatment. Only when a more active attitude to the treatment of uremic patients is held by doctors in general will it be considered absurd to issue a pension to a

patient with a remediable disease. When the preoperatively pensioned patients were compared to a group of patients who had been on continuous sick leave postoperatively or had received a pension after the operation, some differences were found. The former patients represented a higher mean age a longer preoperative time on sick-leave (including the preoperative time on pension) and a preponderance of pyelonephritis compared to the latter group of patients. Pyelonephritis and polycystic kidney disease are usually slowly progressing diseases and those patients especially must be encouraged to continue their work as long as possible. A decision regarding pension has to be postponed until the possibilities of active treatment of uremia have been investigated. Preoperative rehabilitation could start in the preuremic state to avoid the expensive difference between vocational and medical rehabilitation after a successful renal transplantation.

As regards the second question—Why were the pensions not annulled in patients with high degrees of medical rehabilitation?—a number of explanations seem plausible. In essence psychological reasons on the part of the patient and varying degrees of unemployment in the labour market appear to be the most immediate explanations. As mentioned previously the patient had adapted to being out of work. Economically he would benefit little by returning to his earlier employment if the job was not very well paid. During the protracted period of sick leave and pension he had often lost his old employment. Middle-aged or older subjects often have difficulties in finding new work, especially in districts not heavily industrialized, even if they are healthy. Some patients enter the late postoperative course with some more or less incapacitating sequelae. Some patients received pension after transplantation through certificates issued by doctors unconnected with a renal department, this should be avoided. In order to obtain an optimum postoperative rehabilitation in kidney graft recipients, it is probably necessary to apply rehabilitational considerations already in the early preuremic course throughout the procedures of dialysis, transplantation and postoperative management. It is most urgent that strong support is given to the surgeon or nephrologist in charge of the patient by doctors specialized in rehabilitational medicine in order to secure the best final outcome for the patient.

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CAUSES AND DURATION OF RENAL FAILURE AND DURATION OF WORKING DISABILITY IN DIALYSIS CANDIDATES

A Retrospective Study of 104 Patients

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Abstract. A retrospective study of 104 dialysis candidates at end-stage renal failure has shown that this is rarely caused by pyelonephritis. A proportion of 87% of the patients had confirmed chronic pyelonephritis (chronic urinary tract infection). If chronic interstitial nephritis and pyelonephritis are considered as one group, this constituted 26% of the series. Most patients had glomerulonephritis (53.8%) and reached the end-stage at a younger age than patients with pyelonephritis and chronic interstitial nephritis. The progression from moderate renal failure to end-stage failure was more rapid in patients with glomerulonephritis than in those with pyelonephritis and interstitial nephritis. Working disability had not generated a major problem in this group of patients with severe renal failure.

The planning of countrywide dialysis programmes and international transplantation organizations primarily calls for information on requirements for the active treatment of uraemia. Knowledge is also needed on the proportions of different causes of end-stage renal failure and on the progression rate of renal failure with varying aetiology and varying clinical presentation. Moreover it would be of value to possess information on the working disability of patients with chronic uraemia leading to end-stage renal failure. Programmes for prophylactic measures aimed at the prevention of end-stage renal failure should be based upon the results obtained in studies of these questions and focused on the entities of greatest importance from the quantitative aspect. Exact information on the problems outlined is obtainable only by means of prospective population studies concerned with representative areas, with follow-up examinations over a number of years. Until such studies become available, some useful data

may be derived from this retrospective analysis of dialysis candidates with end-stage renal failure.

MATERIAL AND METHODS

The series consisted of 104 consecutive patients admitted to the Renal Ward for regular dialysis or other kinds of treatment as a consequence of end-stage renal failure during the years 1967-70. Twelve patients died during the period of waiting for dialysis treatment. All the other patients underwent dialysis treatment, and 54 had renal transplant. The diagnosis was based upon renal biopsy, the examination of removed kidneys, or the findings at autopsy.

The group of 56 patients with chronic glomerulonephritis has been divided into those with known episode of acute glomerulonephritis (22 patients) followed by either rapid progression (6 patients) or an intermediate phase of proteinuria and/or haematuria (16 patients), and patients presenting with proteinuria and/or haematuria (19 patients) or with renal failure (15 patients). Chronic interstitial nephritis (18 patients) is defined as chronic renal disease with small contracted kidneys and interstitial infiltration of mainly mononuclear cells. Chronic pyelonephritis (9 patients) was defined as chronic interstitial nephritis with history of urinary tract infection, asymmetrically contracted kidneys, and characteristic X-ray findings. The miscellaneous group comprises patients with primary hypertensive disease (4 patients), nephropathy of pregnancy (4 patients) and congenital malformations (5 patients). Fig. 1 indicates the distribution of the patients by age and sex.

End-stage renal failure was defined by consideration of the clinical state, that is, no level of glomerular filtration rate was set. The chemical symptoms of uraemia, gastrointestinal, haematological and neurological, were taken into account. At the moment of end-stage renal failure, defined in this way, the highest endogenous creatinine clearance was $12.6 \text{ ml/min/1.73 m}^2$ (8 but four patients had clearance also below $10 \text{ ml/min/1.73 m}^2$ and in 47 patients it was below $5 \text{ ml/min/1.73 m}^2$). The mean endogenous creatinine clearance was 7.0 ± 3.4 (S.D.) ml/

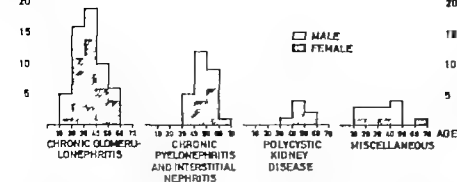
NUMBER
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Fig. 1. Dialysis candidates with end stage renal failure distributed according to underlying disease, age and sex.

mean 173 mg . The mean serum creatinine was 140 ± 37 (S.D.) $\text{mg}/100 \text{ ml}$. Serum creatinine values ranged between 6.1 and $25.0 \text{ mg}/100 \text{ ml}$.

The records of all patients were studied in detail. I most cases information on the phases of the disease was available in the files of the referring hospital.

I most patients the progression of renal disease had been followed up and it was possible to estimate the time of the establishment of renal failure and the earliest elevated serum creatinine value. Whenever possible, calculation was made of the time lapse between the diagnosis of renal failure and end-stage renal failure; in some cases fairly long asymptomatic phase of renal disease was followed by the sudden onset of end-stage renal failure and no renal failure period existed.

For 97 patients data on working disability were available. The period of continuous disability before the end stage was considered, that is, the period of disability due to renal failure. Earlier short periods of disability by reason of hospitalization or other causes, were not included.

RESULTS

Causes of end-stage renal failure

Fig. 1 indicates the causes of end-stage renal failure in this series. In some instances difficulty

Table I. Clinical data on 9 patients with end-stage renal failure arising from chronic pyelonephritis

Pat. no.	Age (y)	Sex	Possible underlying cause
1	32	♀	Hypoplasia of right kidney
2	36	♀	Hypoplasia of left kidney
3	39	♀	Hypoplasia of left kidney
4	43	♀	Aplasia of right kidney
			Hydronephrosis of left kidney
5	55	♂	Nephrolithiasis Ureteric obstruction
6	45	♀	Analgesic abuse
7	56	♀	Childhood pyelonephritis
8	40	♀	No apparent cause
9	50	♀	No apparent cause

was experienced in drawing a definite distinction between pure chronic interstitial nephritis and patients with additional chronic urinary tract infection defined as chronic pyelonephritis. Careful analysis enabled the separation of 9 patients with chronic pyelonephritis. Data on the possible underlying causes in these patients are presented in Table I.

Of the 18 patients with chronic interstitial nephritis 14 had been heavy abusers of analgesics. Two patients had had a history of acute renal failure several years earlier. In 2 patients no evident aetiology was known.

Duration of apparent renal failure

The duration of renal failure from the time when it was first noticed up to the point of end-stage failure, could be calculated in respect of 90 patients. In 6 patients an episode of glomerulonephritis was followed by a rapidly progressive renal failure and the end-stage was reached within 1-18 months. In one patient acute glomerulonephritis was followed by an asymptomatic period of 33 years, with slight proteinuria and/or haematuria before sudden exacerbation and end-stage failure. Furthermore 3 of the glomerulonephritis patients and 3 of those with interstitial nephritis displayed end-stage failure after an episode of oliguria.

Tables II and III indicate the duration of the renal failure period in the different groups of patients.

Table IV illustrates the correlation of the renal failure period with the earliest elevated serum creatinine value in the patients with glomerulonephritis and with chronic interstitial nephritis and pyelonephritis.

Table II. Duration of renal failure period in dialysis candidates with glomerulonephritis, chronic interstitial nephritis and pyelonephritis as cause of end-stage renal failure

	N	Serum creatinine (mean \pm S.D.) at detection of renal failure (mg/100 ml)	Renal failure period (mo.)	
			Median	Range
Acute glomerulonephritis, subsequent period of proteinuria and/or haematuria	15	3.7 ± 2.2	10	3-120
Glomerulonephritis presenting with proteinuria and/or haematuria	16	4.0 ± 2.5	12	2-76
Glomerulonephritis presenting with renal failure	15	3.6 ± 2.1	31	4-80
Chronic interstitial nephritis and pyelonephritis	24	4.0 ± 2.8	48	6-130

Duration of working disability

The duration of working disability as a consequence of renal failure in 97 dialysis candidates is shown in Table V. In grouping the patients according to the duration of disability it is observable that about one half of them, 37 (38.2%) had been working up to the time of end-stage renal failure. Nineteen patients (19.6%) were disabled for more than 10 months before end-stage failure.

About one half of the patients engaged in heavy work, agricultural, manufacture and transport, were disabled for more than 4 months, while more than one half of those doing light work were disabled for less than 4 months.

DISCUSSION

Chronic glomerulonephritis was the predominant cause of end-stage renal failure in this series, a

finding in agreement with previous reports (3, 4, 11). Confirmed chronic pyelonephritis (chronic urinary tract infection) was represented by only 9 patients (8.7%), a figure slightly less than that quoted in series reported earlier (4, 5, 11). Stress needs to be laid upon the difficulties involved in distinction between what is here termed chronic interstitial nephritis and what is usually called chronic pyelonephritis. If the interstitial nephritis group is considered as a whole it represents 26% of the series, a frequency compatible with previous reports.

The low representation of chronic pyelonephritis in this and other series of patients with end-stage renal failure is contrary to the relatively high proportion of a pyelonephritis as a cause of renal failure and death from renal disease (1, 7). This could be explained in many ways. First of all, chronic pyelonephritis and chronic interstitial

Table III. Duration of renal failure period in dialysis candidates with various renal diseases as cause of end-stage renal failure

		Serum creatinine (range) at detection of renal failure (mg/100 ml)	Renal failure period (mo.)
	N		
Polycystic kidney disease	7	1.6-4.3	12-60
Malformations	5	3.1-14.2	8-41
Nephropathy of pregnancy	4	1.4-3.5	12-60
Renal vascular disease hypertension	4	2.0-4.6	2-108

Table IV. Correlation between the earliest elevated serum creatinine value and renal failure period up to end-stage renal failure

Disease	Serum creatinine (mg/100 ml)	N	Period of renal failure (mo.)	
			Median	Range
Glomerulonephritis	2.4	11	31	1-120
	2.5-4.9	17	12	2-96
	5.0	11	4	2-17
Chronic interstitial nephritis and pyelonephritis	<4.9	37	48	6-130
	≥ 5.0	7	36	13-51

Table V Duration of disability arising from renal failure in 97 dialysis candidates

	Occupational group															
	Technical, administrative, managerial and sales				Agricultural				Transport and communications, manufacture				Services, housewives			
Duration of disability (mo.)	None	1-4	5-9	10-	None	1-4	5-9	10-	None	1-4	5-9	10-	None	1-4	5-9	10-
No. of pts.	21	9	9	8	2	1		4	5	4	6	7	9	5	3	4
Total	47				7				22				21			

nephritis lead to end stage renal failure at a much higher age than does chronic glomerulonephritis (7). This has also been shown by the present series. If patients of more than 50 years of age are analysed the group contains only a few patients with glomerulonephritis and about one half of those with chronic pyelonephritis and interstitial nephritis. As only a few patients of more than 50-60 years of age have as a rule been accepted and/or referred to dialysis treatment, it clearly follows that chronic pyelonephritis and chronic interstitial nephritis are relatively infrequent in a series such as the present one and in those previously reported (3-11).

Another fact that influences the low frequency of chronic pyelonephritis and interstitial nephritis among dialysis candidates is the apparent slow progression of renal failure in most patients with these diseases. As was observed here, even in

of high serum creatinine values the end-stage was reached much later than in glomerulonephritis. The slow progression of renal failure not only produces end-stage failure mainly in high age groups, but also makes the active treatment of uraemia less urgent and the opportunities for conservative treatment better.

In only 3 of the 9 patients with chronic pyelonephritis was an apparent underlying cause lacking. This implies that these are the only patients in whom primary non-obstructive pyelonephritis can be regarded as the probable cause of end-stage renal failure. Emphasis must be laid on the fact that, in one of the cases that was probably primary the disease followed a childhood episode of urinary tract infection, and in none of the patients could a history of asymptomatic bacteriuria be confirmed. This finding is in close agreement with that pointed out by

Schechter et al. (11) and reported earlier by others (9).

The present finding confirms that pyelonephritis is a rare cause of end-stage renal failure, even in dialysis candidates whose diagnoses are made on both histological and clinical grounds. The present finding has implications in regard to the efforts that should be made to reduce end-stage renal failure and the need for dialysis treatment and kidney transplantation. The benefits of large and expensive screening programmes for the detection and treatment of bacteriuria should be evaluated by prospective studies, and of course such programmes may prove to be extremely important in the diminution of morbidity figures. However the low frequency of uncomplicated pyelonephritis among dialysis candidates indicates that no important effect upon the reduction of end-stage renal failure is likely to derive from such means. On the other hand the finding of underlying causes in most cases of pyelonephritis clearly emphasizes the need for early detection of urinary tract malformations and the importance of antibacterial treatment and follow-up examination in such cases. The group of patients with chronic interstitial nephritis, in whom urinary tract infection was not diagnosed, indicates that analgesic abuse and acute renal failure are potential causes of end-stage renal failure. Prophylactic measures for the reduction of these fall within achievable limits. Nevertheless the main prophylactic efforts should be focused on glomerulonephritis, which produces so many young dialysis candidates. Development of measures to prevent the disease and its progression to end-stage failure is necessary.

The few cases of acute glomerulonephritis, followed by a rapid progression, have again borne

out the extremely bad prognosis of this category equivalent to what has been termed malignant glomerulonephritis and the rapidly progressive form of glomerulonephritis (6). As renal failure was detected at approximately the same mean levels of serum creatinine in those with glomerulonephritis and those with interstitial nephritis, some comments are justified on the period of apparent renal failure preceding the end-stage. The renal failure period was found to be longest in interstitial nephritis. In fact, every nephrologist knows from experience that this disease has a slow progression and it has been demonstrated that the discontinuation of analgesic abuse, and successful antibacterial treatment, may arrest the progression of renal failure and even allow improvement in function (2, 8, 10). The median renal failure period was shorter in those glomerulonephritis patients who had presented with acute glomerulonephritis, or who prior to renal failure were known to have had proteinuria and/or haematuria, than in those who presented with renal failure. The finding may be accidental, but may reflect a different progression of renal failure in glomerulonephritis with various clinical presentation.

Correlation of the initial elevated serum creatinine level with the renal failure period clearly demonstrated the difference in the progression of renal failure in glomerulonephritis and interstitial nephritis. The finding may have some implications in clinical work, especially in regard to listing dialysis candidates in the order of urgency for active treatment.

This study has further demonstrated that working disability in patients with severe renal failure is not a major problem, about one half of the patients were working up to the time of end-stage failure.

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MULTIPLE MYELOMA WITH URINARY EXCRETION OF HEAVY CHAIN COMPONENTS OF IgG AND NODULAR GLOMERULOSCLEROSIS

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Abstract. A case of multiple myeloma with urinary excretion of considerable amounts of Fc fragments of IgG together with normal glomerular lesions is reported. From the time of discovery of his disease the patient had heavy proteinuria, but renal function was otherwise all preserved and renal biopsy showed normal glomeruli. Over the course of 10 months severe renal insufficiency developed and death from uremia ensued. The urinary protein consisted mainly of Fc fragments from IgG and only small amounts of light chain fragments could be demonstrated. Kidney biopsies and autopsy revealed development of large mesangial nodules in the glomeruli, resembling the lesions in diabetic nodular glomerulosclerosis. Amyloid could not be demonstrated in these nodules.

In about 35% of patients with multiple myeloma Bence Jones protein, which represents the light chains from immunoglobulin molecules, is found in urine when examined by the heat test. In some cases of multiple myeloma other fragments of the immunoglobulins are excreted in the urine. Titus Pruzansky and Ogrzylo (15) found M-components other than Bence Jones protein in the urine of 7% of a series of 157 patients with multiple myeloma. In another series of 30 patients the urine contained Fc fragments in 3 instances and a larger component related to the heavy chain of IgG in another (13). Still other unusual M-components have been reported (12, 17).

In patients with heavy chain disease which was first described in 1964 by Franklin et al. (5) and Oserman and Takatsuki (14) urinary excretion of heavy chains is found, but the urine

from these patients does not contain Bence Jones protein.

We report here our findings in a recently observed case classified as multiple myeloma. This patient had pronounced proteinuria throughout the whole course of his illness. The urinary protein consisted mainly of Fc fragment from IgG and only very small amounts of Bence Jones protein could be demonstrated. In addition this patient developed over a period of a few months severe renal insufficiency with unusual glomerular lesions which have only rarely been described in multiple myeloma.

CASE REPORT

First admission (March 1970)

The patient, a 54-year-old man, had formerly been in good health. He had been admitted because of marked proteinuria. Apart from slight dyspepsia he felt perfectly well.

Physical examination revealed no abnormalities. The liver and spleen were not enlarged and no lymphadenopathy was found. B.P. was normal.

Laboratory investigations showed severe proteinuria and throughout his stay in hospital the urine contained 8-10 g protein/4 h. Because of the electrophoretic pattern and its precipitation on heating to 50°C the protein was initially classified as Bence Jones protein. Apart from this no signs of tubular or glomerular proteinuria were found on urinary electrophoresis.

The first paper-electrophoretic investigation of serum showed a slightly decreased content of IgG. All the other fractions were normal. Serum immunoelectrophoresis showed a weak nonreactive paraprotein (Fig. 2) and slight decrease in IgG and IgM. IgA was normal.

Sternal bone marrow examination revealed 40% plasma cells with signs of immaturity. There was no eosinophilia. ESR was 16-26 mm/h. The white cell and differ

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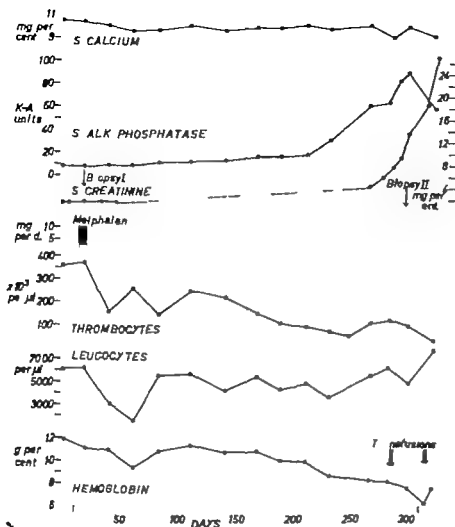


Fig 1 Clinical course of the patient during his illness.

Redial counts were normal and slight normochromic and normocytic anemia was present.

Serum creatinine was 1.2 mg/100 ml and the creatinine clearance 80-100 ml/min. The urinary sediment contained a few leucocytes and hyaline casts.

X-ray examination of the skeleton did not demonstrate osteolytic bone lesions.

Treatment with melfalan was initiated. A few days later bone marrow suppression with leucopenia, thrombocytopenia and aggravated anemia developed. After a short pause in the melfalan administration the WBC returned to normal and remained normal throughout the course of the disease. However the thrombocytopenia and anemia persisted. There was no bleeding tendency.

The patient was discharged for further observation in the Out-patient Department.

First period in the Out-patient Department (April-Nov 1970)

The patient's condition slowly deteriorated and he suffered from increasing tiredness, nausea and diarrhea.

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Kidney function gradually decreased and the serum creatinine rose from 1.3 mg to 4.2 mg/100 ml.

Second admission (Nov-Dec 1970)

The patient was readmitted because of progressive renal insufficiency with malaise, anorexia and vomiting. Physical examination revealed marked pallor but otherwise no differences from the findings at first admission.

Serum and immunoelectrophoresis were unchanged from the first admission and urine electrophoresis still showed excretion of paraprotein, but evidence of glomerular proteinuria was now also present.

During this second period in hospital the creatinine clearance decreased from 11 ml to 4 ml/min. The Hb concentration was 7.5 g/100 ml. Fasting blood glucose concentration and the oral glucose tolerance test were normal.

The serum calcium was normal, but serum phosphorus and alkaline phosphatase were greatly elevated. X-ray examination still did not reveal osteolytic bone lesions.

The treatment with melfalan was continued.



Fig. 2 Immunoelectrophoretic analyses of the serum.

(1) Developed with anti-human serum. The patient's serum is applied below the trough. Note the faint re-duplication of the IgG precipitin arc.

(2) Developed with anti-IgA. The patient's serum is applied above the trough.

(3) Developed with anti-IgM. The patient's serum is applied above the trough.

Third admission (Jan. 1971)

Two weeks after discharge he was readmitted severely anemic. His condition deteriorated rapidly and he died 9 days later.

ANALYSIS OF URINARY PROTEINS

Isolation of the abnormal protein from the urine

Each urinary sample comprised a 24-hour sample. After centrifugation sodium azide was added as preservative. The samples were concentrated in an Amicon Ultrafiltration cell using Daclo Ultrafiltration membranes, type UM 10. (The membrane retains substances with molecular weights in excess of 10 000.) The concentrated sample was dialysed against potassium phosphate buffer pH 7.8, in Visking tubing (23/32 inflated diameter). After dialysis the concentrated urine was either directly applied to an ion exchange chromatography column or stored at -20°C .

Ion exchange chromatography was performed in columns with DEAE-Sephadex A 50 in potassium phosphate buffer at pH 7.8. Gradient elution was done with increasing ionic strengths of potassium phosphate, pH 7.8. The protein fractions were then placed on a Sephadex G-100 column and eluted with sodium phosphate buffer pH 7.0.

Determination of molecular weights was performed by gel filtration on Sephadex G-100 (24).

Protein electrophoresis was accomplished on cellulose acetate using barbital buffer, pH 8.6.

Immunological techniques. The following antisera from Behringwerke were used. Anti-human serum (AHIS), anti-Bence Jones kappa, anti-Bence Jones lambda, anti-Fc fragment, anti-Fd fragment, anti-Fab fragment.

Anti-lambda and anti-kappa sera from Biorad were made specific for free light chains by absorption with Cohen fraction II (21).

Immunoelectrophoresis was performed using modification of Scheidegger's method (18).

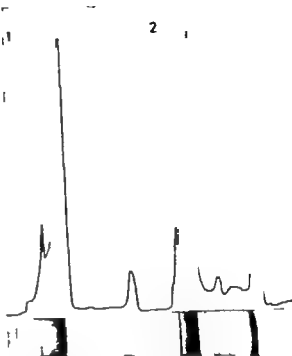


Fig. 3 Cellulose acetate electrophoretic patterns of the urinary protein.

(1) Urine, May 1970, protein concentration 0.8 g/100 ml. Almost all the protein is present in the γ -zone.

(2) Urine, Jan. 1971 protein concentration 4.2 g/100 ml. In addition to the abnormal γ -spike an increased excretion of albumin has appeared.

Oxydative isoelectric focusing was carried out in medium of 1% Agarose Lhas, containing barbital buffer pH 7.2, and with an ionic strength of 0.06. The plates were stained with brilliant coomassie blue.

Ultracentrifugal analysis was performed by C. Christensen, Institute of Medical Microbiology University of Aarhus, using Beckman Model E analytical ultracentrifuge.

RESULTS

Cellulose acetate electrophoresis of urine collected during the first hospitalization showed that most of the urinary protein moved with the γ -fraction. When the patient later became uremic, the electrophoretic pattern changed, the urine contained considerable amounts of albumin, giving a pattern of glomerular proteinuria (Fig. 3).

The protein with γ -mobility was eluted from DEAE Sephadex in a very narrow peak between 0.03 and 0.05 M potassium phosphate buffer. Further purification was carried out on a Sephadex G-100 column. Here the γ -fraction was eluted as a single, very sharp zone.

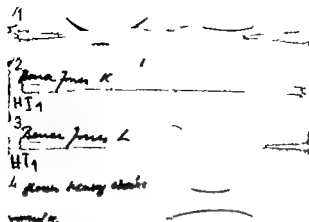


Fig. 4 Immunoelectrophoretic analyses of the abnormal urinary protein.

(1) Developed with anti-human serum. Concentrated urine from the patient is applied above the trough.

(2) Developed with anti-kappa type light chain serum. Purified abnormal protein from the patient is applied below the trough.

(3) Developed with anti-lambda type light chain serum. Purified abnormal protein from the patient is applied below the trough.

(4) Developed with anti-heavy chain serum. The purified abnormal protein from the patient is applied above and normal serum below the trough.

Cellulose acetate electrophoresis of this purified protein showed a slightly diffuse fraction in the fast γ -zone. The heat test for Bence Jones protein was positive. Molecular weight was estimated to be 47 000.

Immunoelectrophoresis of the patient's serum developed with AHS showed an abnormal protein as a weak precipitin arc closer to the trough than the otherwise normal-looking IgG precipitin line. Immunoelectrophoresis of the purified abnormal urinary protein developed with antiserum to Fc fragment revealed a clear precipitin line (Fig. 4). The mobility was identical with the position of the precipitin arc of the abnormal protein in serum.

The absence of a normal-looking IgG precipitin line in the concentrated urine (Fig. 4) in spite of a normal IgG precipitin line in serum and the presence of the protein in high concentration in the urine suggests the relatively smaller molecular size of the protein.

Immunoelectrophoresis developed with absorbed anti-lambda serum showed only a trace of lambda chains (Fig. 4). The absence of a reaction with the Fc fragment suggests that the

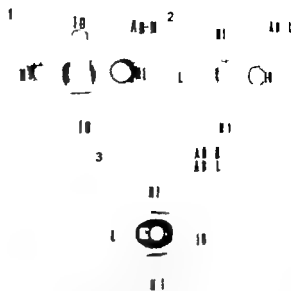


Fig. 5 Ouchterlony double diffusion analysis.

(1) The analysis shows reaction of identity between the purified abnormal urinary protein from the patient (HT) and IgG (IG). In center well anti-Fc serum.

(2) Reaction of identity is seen between the purified abnormal protein from the patient (HT) and free lambda-type light chains (L). The experiment also shows that light chains bound to heavy chains in intact IgG (IG) do not react with the antibody in center well: antiserum to free lambda chains.

(3) Ouchterlony analysis developed with mixture of antisera against free lambda type light chains and Fc fragment (center well) reveals two precipitin lines between the antisera and the purified protein (HT), showing the presence of two different fragments of IgG in the serum.

was not precipitated by anti-kappa or anti-Fc fragment sera.

On Ouchterlony analysis the protein gave a reaction of identity with Cohn fraction II when tested against antiserum to Fc fragment (Fig. 5) and a reaction of partial identity when tested against antiserum to IgG. On diffusion against absorbed anti-lambda serum a line of identity appeared between the purified abnormal protein and a standard of lambda chains. Fig. 5 shows further results of immunodiffusion of the purified protein against a mixture of antisera to Fc fragment and free lambda chains. The appearance of two precipitin lines shows the presence of two fragments of IgG inseparable by DEAE filtration one with determinants from lambda chains and one with determinants from Fc fragment. The absence of a reaction with the Fc fragment suggests that the

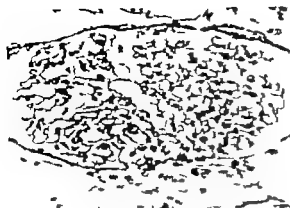


Fig 6. Normal glomerulus from the first kidney biopsy PAS-staining 174.



Fig 7. Glomerulus with mesangial nodules. Second kidney biopsy PAS-staining 174.

protein with heavy chain determinants was probably the Fc fragment.

The sedimentation coefficient was estimated as $S_{20} = 3.7$

PATHOLOGY

First kidney biopsy (March 1970)

The percutaneous kidney biopsy contained 15 glomeruli, all of which were normal (Fig. 6). Apart from slight focal tubular atrophy and slight interstitial fibrosis no abnormalities were found.

Using immunofluorescent techniques neither IgA, IgM, IgG, complement nor fibrinogen could be demonstrated in glomeruli, vessels, tubules or interstitium.

Second kidney biopsy (Dec 1970)

This biopsy contained 13 glomeruli. Two of these were completely hyalinized. In 5 glomeruli the mesangial regions were moderately enlarged. In the remaining glomeruli the lobular structure was much more conspicuous than in normals because of the presence of nodular PAS-positive mesangial deposits (Fig. 7). Centrally these nodules had fine fibrillar structure and they contained few mesangial cells. Connective tissue staining revealed collagen in the central portion of the nodules. Peripherally thin border of psammomatous material was found. Most of these nodules were surrounded by single row of capillary loops, some of which are partly compressed by the mesangial nodules. Outside the areas with nodular formations the capillary loops were normal, and no hypercellularity was found. Neither capsular drops nor fibrous caps could be demonstrated.

Four different staining reactions for amyloid (methyl violet, Congo red, thioflavin T and amyloid staining with uric acid F3B) did not demonstrate amyloid in these glomerular deposits.

A moderate, diffuse tubular atrophy was found. Some tubules contained granular or hyaline casts. The tubular epithelial cells often showed non-specific degenerative changes, but intact cell formation around the casts was

not found. A few tubules contained colloid, intracellular crystal-like bodies.

The arterioles were normal. In the wall of at least lobular artery rim slight intimal fibrosis was seen. Amounts of amyloid could be seen. There was moderate, diffuse, interstitial fibrosis and the interstitium contained few small foci of lymphocytes and plasma cells.

Immunofluorescence showed small amounts of fibrinogen in the peripheral part of the mesangial nodules, but no complement, IgA, IgM or IgG was found.

Autopsy (Jan. 1971)

Autopsy revealed pulmonary edema, left-sided D -trans pleurisy and fibrous pericarditis with 100 ml serum fluid in the pericardium. The heart weighed 640 g. The myocardium was hypertrophied with slight diffuse fibrosis. The coronary arteries showed moderate atherosclerosis. The liver and spleen were slightly enlarged. There was no swelling of the lymph nodes.

On macroscopic examination no visible extramedullary tumours were seen in the ribs, sternum, colon or left femur.

Macroscopic investigation of sternum, lower and colon revealed diffuse plasmacytoma in the bone marrow which in some places contained small solid tumours with an appearance similar to typical myeloma. There was no plasma cell infiltration or amyloid in the lymph nodes, liver or splenic pulp.

Each kidney weighed 200 g. Both kidneys had the same appearance with pale, reddish-yellow broadened, firm cortex, all demarcated from the somewhat friable medulla. The renal pelvis and vessels were normal.

Histologic examination of the kidneys showed changes of the same type as observed in the second kidney biopsy. About 90% of the glomeruli contained several mesangial nodules.

Small amounts of amyloid were found in the interstitial connective tissue of the myocardium, and a few small arteries in the lungs, spleen, suprarenal gland and submucosa from stomach and small intestine. In the kidneys traces of amyloid were seen in the few interlobular arteries, but amyloid could not be

strated in glomeruli, arterioles, capillaries or interstitium. Several different staining reactions did not reveal fibrin, neutral fat, phospholipids or acid mucopolysaccharides in the glomerular nodules. Neither could heavy chains be demonstrated by immunofluorescence using an antiserum against heavy chains from IgG.

DISCUSSION

It has been shown that urine from healthy persons normally contains small amounts of Fc fragment (0.19 mg/24 h) from the heavy chains of IgG molecules (23). Most or possibly all the Fc fragment excreted derives from the catabolism of IgG. Decomposition of IgG leading to formation of Fc fragments probably does not take place in urine (3).

The synthesis of light and heavy chains in normal plasma cells is a well balanced process. There is normally however a slight overproduction of light chains, which results in the urinary excretion of slight amounts of light chains (3-4 mg/24 h). Thus these light chains naturally occurring in the urine originate from the synthesis of γ -globulins in contrast to the Fc fragments.

In multiple myeloma there is a greater or lesser production of abnormal "whole" immunoglobulins and light chains (= Bence Jones protein) resulting in the occurrence of a so-called M-component in the serum and eventually Bence Jones proteinuria.

By means of the numerous specific antisera which have become available during recent years, it has been possible to show in some cases of multiple myeloma that M-components other than Bence Jones protein and eventually "whole" immunoglobulins can be found in the urine.

In the present case it was not possible to demonstrate light chains in the urine until it had been concentrated. However by immunoelectrophoresis and immunodiffusion a strong reaction was found between the urinary protein and antiserum against the Fc fragment of heavy chains from IgG.

The molecular weight of the excreted protein was 47 000 corresponding to the molecular weight of the Fc fragment. At elution the protein appeared in a single peak, containing both lambda chain determinants and Fc fragment determinants. The elution curve did not show signs of polymerization.

Because of polymerization Bence Jones protein may occur as monomers (20-25% of the total quantity of light chains) dimers (75-80%) and

tetramers (1-2%) with molecular weights of 22 000 44 000 and 88 000 respectively (1). Therefore, Bence Jones protein is eluted in 3 peaks. In our case the elution curve gave no evidence of polymerization. This, together with the difficult immunologic detection of lambda chains, indicates that the light chains formed only a small part of the urinary protein.

On Ouchterlony immunodiffusion two precipitin arcs were found when a mixture of antiserum to lambda chains and Fc fragment was used. This indicates that the patient excreted two separate proteins and not a single protein consisting of light chains attached to a heavy chain fragment, which situation has been described earlier (17).

In the ultracentrifuge the protein sedimented homogeneously with $S_{20,w} = 3.7$ corresponding to the value for the Fc fragment (14). Similar values for monomers, dimers and tetramers of Bence Jones protein are 2.0 3.6 and 5.5 (7).

Thus, in the present case there was a pronounced urinary excretion of Fc fragments from heavy chains. However neither the pattern of proteinuria nor the clinical course was typical of heavy chain disease. The latter is characterized by loss of weight, recurring pneumonia, anemia, eosinophilia, painful lymphadenopathy and urinary excretion of heavy chains or fragments of them without simultaneous excretion of light chains. Osteolytic lesions are not found.

In the present case heavy proteinuria and a gradual reduction of renal function were initially observed. Then after several months, severe renal insufficiency developed over the course of 6 weeks, leading to death from uremia.

At no time did the patient have lymphadenopathy and autopsy did not disclose significant changes in the lymph nodes.

Even though it had not been possible to detect osteolytic bone lesions radiographically during life, histologic examination of bone marrow removed at autopsy showed small myelomas with slight destruction of the surrounding bone trabeculae. In addition a diffuse plasmacytosis was found in the marrow and in our opinion this case should be classified as one of multiple myeloma.

The immunologic investigations of the patient's urine were done after his death on samples collected for other purposes during the last two months of life. The specific type of proteinuria was not identified *in vivo* and unfortunately serum

samples were not available for analysis with special antisera.

Throughout the whole course of the patient's illness ordinary paper electrophoresis of serum revealed a normal pattern without indication of M-components. Serum immunoelectrophoresis, using AHS anti-IgM, and anti-IgA, showed a faint branching of the IgG arc. In our experience such branching has only been observed in patients with plasmocytic neoplasms. This branching of the IgG arc in the serum immunoelectrophoresis was found throughout the course. This may indicate that treatment with melphalan was ineffective, as may the constant heavy urinary excretion of paraprotein. Lack of response is rather common in the more malignant cases of multiple myeloma (9).

The very small amounts of paraprotein in the serum and the constant heavy urinary excretion indicate that the abnormal protein had a relatively low molecular weight. Therefore, the undifferentiated plasma cells were probably not able to produce whole immunoglobulins. As it has only been possible to examine urine samples taken during the last two months of life, we do not know whether the patient excreted both Fc fragments and light chains from the beginning of his illness.

The cause of death was uremia. This is not unusual in multiple myeloma, as about 40% of patients with this disease die of uremia (2). Renal abnormalities are common in multiple myeloma. Usually the main lesions are found in the tubules and interstitium, and in many patients the well known "myeloma-kidney" is found, the most prominent components of which are the presence of dense homogeneous, eosinophilic casts in dilated distal and collecting tubules. These casts are frequently surrounded by giant cells. Moreover there is well pronounced tubular atrophy and diffuse interstitial fibrosis. On the other hand, glomerular lesions are inconspicuous. Usually one only finds a slight thickening of the capillary walls and eventually a moderate enlargement of the mesangial regions (8, 22).

Only a few publications mention more severe glomerular lesions in multiple myeloma. Kobornick and Whiteside (11) reported on glomerular lesions in 13 cases of myeloma. In 11 of these 13 patients they found moderate thickening of the glomerular capillary walls and widening of the

mesangial regions; however a photomicrograph from their third case shows occasional nodular changes in a glomerulus, and the authors mention that this lesion resembles diabetic capillary glomerulosclerosis; the patient did not have diabetes. This photomicrograph does resemble the glomerular changes in our case.

Fisher et al. (4) have published a light and electron microscopic study of glomerular lesions in 7 cases of multiple myeloma. One of these patients had diabetes mellitus. In the other 6 patients the glomeruli showed a moderate enlargement of the mesangial regions. This paper does not mention whether some of the patients excreted heavy chains or fragments thereof.

Rosen et al. (16) reported on a patient with multiple myeloma and nephrotic syndrome. Light microscopy of a kidney biopsy revealed enlarged glomeruli with accentuated lobulation and mesangial foam cells. Ultrastructurally the mesangial matrix was markedly increased and contained numerous small, densely osmophilic areas.

Fries et al. (6) also found subendothelial and membranous and mesangial glomerular lesions in a case of multiple myeloma.

In a recently reported case of multiple myeloma with severe renal insufficiency Scherz et al. (17) and van Haelst (19) found heavy renal glomerular PAS-positive deposits, mostly located intracapillary. They were negative on silver and fibrin staining. Ultrastructurally they consisted of osmophilic fibrils.

Kaplan and Kaplan (10) have reported on a case of monoclonal gammopathy and nephrotic syndrome. This patient had rapidly progressive renal failure. Renal biopsy revealed a chronic proliferative glomerulonephritis with marked endothelial proliferation quite unlike the findings in our case.

The two kidney biopsies from our patient showed that peculiar glomerular lesions had developed over the course of 10 months. Morphologically these lesions may be characterized as nodular glomerulosclerosis or—in respect of some of the details—lobular glomerulonephritis because of the occurrence of mesangial nuclei in some of the nodules. Hyalinization of the arterioles, intrarenal seen in diabetic glomerulosclerosis, was not found in our case. Diabetes mellitus could be ruled out clinically.

We cannot explain the

glomerular lesions in our patient, but hypothetically it could be imagined that the mesangial nodules resulted from ultrafiltration of considerable quantities of Fc fragment from serum with subsequent stimulation of mesangial basement membrane synthesis. Probably the nodules are not simple deposits of amyloid or heavy chain from IgG because of the negative results of special stainings and immunofluorescent investigation with antiserum against heavy chains.

ACKNOWLEDGEMENTS

This study was supported by grants from Statens Lægevidenskabelige Forskningsråd and Røntgenrengøring til Gigtens Bekæmpelse.

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THE GLOMERULAR FILTRATION RATE DURING MODERATE HYPERGLYCEMIA IN NORMAL MAN

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Abstract. At ten examinations the renal clearances of inulin and ^{51}Cr -EDTA have been determined before, during and after hyperglycemia induced in nine normal young men. The blood glucose concentration was kept moderately elevated for period of 2 to 3 hours by combined oral and intravenous administration of glucose. During the hyperglycemia the inulin and ^{51}Cr -EDTA clearances showed highly significant increase averaging 14 and 13% respectively. The urinary sodium excretion rate was reduced during this hyperglycemia. Five subjects were examined during water load. No significant change occurred in the glomerular filtration rate (GFR). A possible explanation of the effect on the GFR of glucose load could be an interaction between the glucose and sodium transport in the kidney combined with a moderate increase in the plasma volume.

It has recently been shown that in young diabetics without nephropathy the glomerular filtration rate (GFR) is higher than normal (12, 13, 25-29). In order to examine the influence of moderate hyperglycemia on the GFR, the renal clearances of inulin and ^{51}Cr -EDTA were determined in normal subjects under glucose load. Some of the present results have been published in a preliminary report (8).

MATERIAL AND METHODS

Material

Nine normal men, aged 22-30 years, volunteered for the glucose studies. In one of them the examination was repeated after 4 months (nos. 37 and 61). The control material consisted of five normal men belonging to the same age group. Two of them also participated in the glucose experiments (nos. 37, 148 and 72, 146).

General procedure

The examination lasted from 8.30 a.m. to 3 p.m. The subject was confined to bed and had fasted overnight. When the examination started, the subject drank 0.75 l water and thereafter about 0.5 l water every hour. He

did not drink during the I.V. administration of glucose. The bladder was emptied by spontaneous voiding. The subject stood up during emptying of the bladder. Blood was sampled from a Viggo catheter placed in veins in the forearm, and capillary blood samples were taken from the ear.

Experimental procedure (Fig. 1)

Following three 30-min control periods, 1 g glucose/kg b.wt. was given orally. In seven cases (nos. 64-81) an additional infusion (5-10 ml 5% glucose/min) during 90 min was given 45-60 min later. During hyperglycemia there were from four to six 30-min clearance periods and after hyperglycemia from one to three. The duration of the hyperglycemia is here defined as the interval between the oral glucose administration and the end of the clearance period during which the blood glucose concentration fell below the fasting value.

Analytical procedure

The renal clearance of inulin and of ^{51}Cr -EDTA was determined by the continuous infusion technique (7). The first clearance period started approximately 60 min after administration of the priming dose and cessation of sustaining infusion. Clearance was calculated according to the formula $Cl = (U/V)/P$ where U is the urine flow per minute, V the concentration of the clearance substance in urine, and P the concentration in serum. P was obtained by interpolating the serum concentration determined at the middle of the clearance period minus 2 min. The inulin concentration in serum and in urine was measured according to Boyesen method (4). Before the analyses, inulin and glucose in serum were separated by gel filtration (G-25 Sephadex in H_2O). In urine samples with glucose the inulin concentration was corrected by employing the inulin equivalent for glucose (1.22%). The activity of ^{51}Cr in serum and urine was measured in a well type scintillation detector. At least 10 000 counts were recorded. The plasma volume (PV) was determined with T_{100} during the last control period. The formula employed for its calculation was $PV = (A/0.93) \cdot C$ where A is the I.V. injected amount and C the plasma concentration 15 min after the injection. The factor 0.93 was used on the assumption that after 15 min 2% of the amount of T_{100} had left the intravascular space. The T_{100} con-

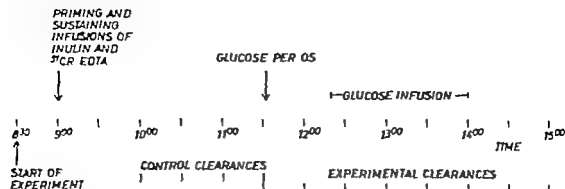


Fig. 1 Flow sheet of glucose experiments.

centration was measured by means of Torenberg method (37). The colloid osmotic pressure (COP) was determined in all serum samples with a Tybjerg Hansen osmometer (31).

The capillary glucose concentration was measured with glucose oxidase in an Auto-analyser at least once during each clearance period. The glucose concentration in urine was measured with glucose oxidase after dilution in the ratio 0.5:9 (8 ml Homogy's zinc sulphate + 1 ml 0.75 N sodium hydroxide). The sodium concentration in plasma and urine was determined with flame photometer.

Control experiment

With the following exceptions the procedure was the same as in the glucose studies.

1) The subjects did not fast overnight, but had light meal approximately one hour before the examination started. 2) There was no infusion of glucose. 3) The insulin clearance was not determined. 4) The blood glucose concentration in capillary blood was determined only twice during the examination. 5) During the whole examination subjects drank about 0.5 l water every hour.

Table I. Inulin and ^{51}Cr EDTA clearances before (C), during (H) and after (AH) hyperglycemia

Mean = mean value of clearances and blood glucose concentrations in the 4 periods with highest glucose concentrations

Subject no.	Inulin clearance (ml/min)				^{51}Cr -EDTA clearance (ml/min)				Blood glucose (mg/100 ml) M
	C	H	AH	M	C	H	AH	M	
37	—	—	—	—	113	120	103	176	183
61	144	151	146	157	125	134	125	141	173
66	131	155	140	161	117	130	117	135	132
68	137	144	143	151	119	130	131	137	144
69	121	135	116	148	113	127	111	129	144
77	112	125	102	125	100	112	94	113	180
73	147	145	143	151	131	135	127	143	138
74	144	159	147	170	118	130	119	137	194
78	112	122	122	125	106	117	114	118	207*
81	142	155	119	155	118	135	103	136	163
Mean	132	143	131	149	116	127	114	132	166
Significance of difference from control	—	<0.01	>0.1	<0.01	—	<0.01	>0.1	<0.01	—

The statistical analysis was made by employing Wilcoxon's two-tailed test for pair differences.

RESULTS

For the analysis the results were grouped as values before, during and after hyperglycemia. Tables I and II show for each subject the effect of the induced acute hyperglycemia on the inulin and the ^{51}Cr EDTA clearances as well as on the sodium excretion rate. An analysis was made also of the events from clearance period to clearance period. The results are given as average curves in Fig. 2.

Glucose in blood and urine

The average curve of the mean blood glucose concentration is shown in Fig. 1. The hyperglycemia lasted 2 to 3 hours. The blood glucose concentration reached its peak value of 140-

Table II. Urinary sodium excretion ($\mu\text{Eq}/\text{min}$) in the first (C_1) and the last (C_2) control period during (H) and after (AH) hyperglycemia

Subject no.	C	C_1	H	AH
37	78	89	73	107
61	136	315	224	323
64	104	165	116	149
68	149	217	139	198
69	77	255	243	148
72	136	231	136	138
73	83	55	112	130
74	87	154	97	52
78	178	184	91	91
81	198	236	191	208
Mean	123	192	142	156
Significance of difference		<0.02	<0.02	>0.1

250 ml/100 ml in the middle of this interval. In seven cases glucosuria occurred in three or four clearance periods during the hyperglycemia. The excretion rate of glucose was at a maximum 10–60 mg/min.

Inulin and C -EDTA clearances

Corrected for BSA (1.73 m^2) the respective mean values for inulin and ^{51}Cr EDTA clearance before hyperglycemia were 112.7 ± 8.7 (S.D.) ml/min and 99.0 ± 5.6 (S.D.) ml/min. For all the 89 clearance periods the mean ratio ^{51}Cr EDTA clearance/inulin clearance \pm S.E.M. was 0.90 ± 0.01 a finding in accordance with the results of other authors (1, 10, 18, 30). Table I gives the individual mean values of the inulin and the ^{51}Cr EDTA clearance before, during and after hyperglycemia together with the mean values of the clearances and the blood glucose concentrations in the two periods where the latter parameter reached its maximum. In all the subjects the mean ^{51}Cr EDTA clearance rose during the hyperglycemia, and so did the mean inulin clearance except in one subject (no. 73). The clearance values before and after the hyperglycemia did not differ significantly. In the two periods with the highest blood glucose concentrations the increase in the inulin clearance averaged 14% (3–23) and in the ^{51}Cr EDTA clearance 13% (9–16). Fig. 2 illustrates the course of the clearances from period to period during the hyperglycemia. Compared to the last control period the clearances were significantly increased

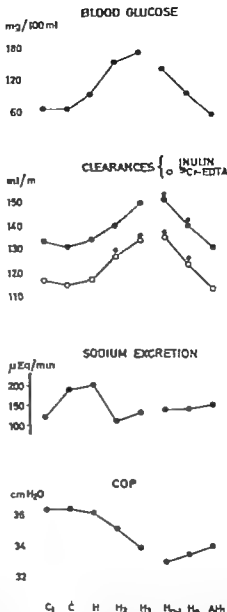


Fig. 2. Mean values of capillary blood glucose concentration, inulin and ^{51}Cr -EDTA clearances, urinary sodium excretion rate and colloid oncotic pressure (COP) in serum in 9 subjects under experimental glucose load. The sign * indicates that the clearance value is significantly increased (p -value at least <0.05) compared with the value in the last control period.

C_1 = first control period, C_2 = last control period, H_{1-3} = first three hyperglycemia periods, H_4 = penultimate hyperglycemia period, AH_1 = last hyperglycemia period, AH_2 = first posthyperglycemia period.

in all the hyperglycemia periods shown, except in the first for both clearances and in the second for the inulin clearance.

Table III ^{51}Cr EDTA clearance and urinary sodium excretion in control experiments

Subject no	^{51}Cr -EDTA clearance (ml/min)			Sodium excretion ($\mu\text{Eq}/\text{min}$)			
	Mean of period 1-3	Mean of period 4-8	Mean of period 9-10	Period 1	Period 3	Mean of period 4-8	Mean of period 9-10
134	111	107	111	231	406	367	421
146	107	104	105	273	328	294	355
148	121	114	106	343	315	277	224
150	98	100	104	376	477	414	448
155	110	109	105	86	256	301	276
Mean	109	107	106	266	356	331	345

Plasma sodium and urinary sodium excretion

The plasma sodium concentration in the last control period averaged 138.8 ± 2.6 (S.D.) mEq/l. There were only small changes in the sodium concentration during the hyperglycemia. The highest and lowest values during hyperglycemia were, on average 139.4 ± 2.6 (S.D.) mEq/l and 137.1 ± 2.4 (S.D.) mEq/l, respectively.

In Table II are listed the individual values for the sodium excretion rate during the first and the last control period together with the individual mean values for the excretion rate during and after hyperglycemia. In all subjects but one (no. 73) the excretion rate rose from the first to the last control period and fell during the hyperglycemia. The course of the excretion rate from period to period during the hyperglycemia is seen in Fig. 2. There was no difference between the excretion rate in the last control period and in the first hyperglycemia period. Compared to this period it was significantly reduced in the second period during hyperglycemia ($p < 0.01$). In the subsequent periods the excretion rate remained almost constant.

Urine flow

The mean urine flows before, during and after hyperglycemia were respectively 11.6 (8.6-17.1) 10.2 (8.1-13.9) and 10.1 ml/min (5.7-18.5).

Plasma volume and COP in serum

Corrected for BSA (1.73 m^2) the mean plasma volume in the last control period was 2.8 ± 0.3 (S.D.) l. In the same period the mean COP in serum was 36.4 ± 3.0 (S.D.) cm H_2O . Fig. 2

shows that COP was decreasing during the first three hyperglycemia periods and that the greatest fall in most cases occurred in the penultimate hyperglycemia period or later. By this period the total fall in COP averaged 9.3% (7-16) corresponding to an average decrease in the total protein concentration in plasma of 6.0% as calculated according to the equation given by Landis and Pappenheimer (21). Using this value, the dilution of plasma corresponds to an average rise in the plasma volume of 0.2 l (0.1-0.4).

Control experiments

The blood glucose concentration in the third and the eighth periods averaged 72 mg/100 ml (61-80) and 65 mg/100 ml (60-72), respectively. The individual values for ^{51}Cr EDTA clearance and urinary sodium excretion rate are given in Table III. The mean values employed originate from the periods 1-3, 4-8 and 9-10 because, with respect to time and duration these intervals correspond to the control, the hyperglycemia and the posthyperglycemia period in the glucose studies. The ^{51}Cr EDTA clearance did not change. The sodium excretion rate rose from the first to the third period and fell slightly during the following periods. The respective mean values for urine flow in the periods 1-3, 4-8 and 9-10 were 12.6 (10.2-14.6), 9.5 (7.8-11.2) and 10.9 ml/min (6.0-12.7). During the whole examination COP in serum showed a fall, totalling on the average 4.2%.

DISCUSSION

GFR during glucose load

The present work shows that the inulin clearance rose significantly in the hyperglycemia period and

subsequently fell to the same value as in the control period. As the ^{51}Cr EDTA clearance changed concurrently while it remained constant in the control experiments, the administration of glucose must be the cause of the changes in GFR.

In previous studies, in which renal function tests in man have been performed during glucose administration, GFR has been found to be unchanged (16, 22, 26, 27) or increased (2, 6, 14, 17, 27). By grouping the given data concerning the blood glucose level, however, there is close correspondence within the groups between the results from the different studies.

During minor elevation of the blood glucose concentration Mogensen (27) recently reported an unchanged GFR determined by ^{125}I iothalamat clearance in normals at a venous plasma glucose concentration averaging 138 mg/100 ml following oral glucose administration. In the present work GFR was probably increased at a blood glucose concentration averaging 150 mg/100 ml in the second hyperglycemia period (^{51}Cr -EDTA clearance significantly increased, inulin clearance increased but not significantly) whereas GFR was significantly increased at a blood glucose concentration averaging 168 mg/100 ml in the third hyperglycemia period (cf. Fig. 2). These findings suggest that the blood glucose concentration must exceed 140 mg/100 ml for a significant increase in GFR to occur.

At somewhat higher blood glucose concentrations GFR in normals has been found to increase during infusion of glucose (6, 14, 17, 27). In the studies by Brod et al. (6) and by Ek (14) an i.v. infusion was given of 1 g and of 0.8–1.5 g glucose/min, respectively. Shortly after the start of the infusion a rise took place in the inulin clearance to total on the average 22 and 27% respectively during the first 50–60 min. In the work by Fox et al. (17) the inulin clearance rose by 10% during infusion of approximately 0.5 g glucose/min, the venous plasma glucose concentration averaging 250 mg/100 ml. Recently Mogensen (27) reported a consistent rise in GFR determined by ^{125}I iothalamat clearance in normals during i.v. glucose infusion, the average venous plasma glucose concentration being 275 mg/100 ml.

At very high blood glucose concentrations, as used when determining the maximum rate of

glucose reabsorption Farber et al. (16) Levitan (22) and Mogensen (26) did not find a significant increase in GFR. In contrast to Aurell et al. (2) The osmolar clearance during the glucose infusion was not measured in any of the latter studies, but a pronounced osmotic diuresis must have been present at the high blood glucose concentrations employed. However the reported data permit a calculation of it. At a mean blood glucose concentration of 650 mg/100 ml the osmolar clearance must have been about 10–12 ml/min in Mogensen's examinations (26) where GFR determined by ^{125}I iothalamat clearance was unchanged. In the studies by Aurell et al. (2) on the other hand, where GFR determined by ^{51}Cr EDTA clearance increased 10% and the blood glucose concentration was about 400 mg/100 ml, it must have been about half this size. During mannitol-induced osmotic diuresis in normotensive patients Baldwin et al. (3) found that, at an osmolar clearance of 10–12 ml/min, GFR was reduced by 15–20% whereas it was but slightly reduced at a lower osmolar clearance. The different effects on GFR under osmotic diuresis of the same order of magnitude suggest that, at high blood glucose concentrations, the GFR-reducing effect of the osmotic diuresis is outweighed by GFR-increasing factors with the result that, dependent on the magnitude of the osmotic diuresis, GFR is either increased (2) or unchanged (26). These GFR-increasing factors may quite well be identical to those responsible for the increased GFR during moderate hyperglycemia demonstrated in the present and in the reported studies (6, 14, 17, 27).

If the same GFR-increasing factors contribute to the high GFR in diabetics, the GFR-reducing effect of osmotic diuresis may partly explain the lack of positive correlation between the blood glucose concentration and GFR in diabetics (12, 25), and it may also explain the fall in GFR when a moderately elevated blood glucose concentration in diabetics is pronouncedly increased either by oral or i.v. glucose administration (16, 26, 27).

Explanations of the increased GFR during glucose load

It was assumed by Brod et al. (6) Ek (14) and Mogensen (27) that the rise in GFR during acute moderate hyperglycemia was due to an expansion of the extracellular fluid volumes in connection

with a reduction of the colloid osmotic pressure in plasma. According to a recent survey by Wesson (37) GFR in man does not increase during acute Lv infusion of isotonic saline unless 45 ml/min and upwards are infused. By infusing prehydrated subjects at such high infusion rates Ladd (20) found a maximum average increase of 22% in the inulin clearance and an average fall of 22% in the plasma protein concentration. In the present work the inulin clearance was significantly increased by 15% in the third hyperglycemia period (cf. Fig. 2). At the same time COP in serum was reduced by 6.9% corresponding to a decrease in the plasma protein concentration of only 4.4% (21). It is therefore unlikely that the rise in GFR during the acute moderate hyperglycemia is caused only by the increase in plasma volume and the reduction in COP. This view is also supported by the results of Fox et al. (17). They found the inulin clearance to increase significantly during infusion of glucose whereas it was unchanged during infusion of xylose and galactose despite the fact that the volume rate of infusion was the same for all three sugars.

During oral or Lv glucose administration to normals a change takes place in the plasma concentration of insulin, the growth hormone, glucagon, free fatty acids, and perhaps also of catecholamines. The possible separate effect of insulin and free fatty acids on GFR is not known, but in normals the injection of glucagon and repeated administration of the growth hormone has been found to increase GFR (11-15). During glucose administration the glucagon concentration in plasma is, however, reduced (28-34) and the concentration of the growth hormone unchanged or reduced (9). The contributory effect of these hormones on the increase in GFR during glucose administration, therefore, is not very probable.

The rise in sodium excretion rate from the first to the last control period in the glucose experiments (Table II) and from the first to the third period in the control experiments (Table III) corresponds to the rise before noon known from the diurnal variation pattern (24-31). The reduction during hyperglycemia (Table II and Fig. 2) was more pronounced than that found in the control experiments. This suggests that the kidney responds to an acute moderate hyperglycemia with a reduced rate of sodium excretion. Ek (14) found that the sodium excretion rate in normal subjects

was unchanged during Lv glucose infusion at a rate of 25-30 ml/min.

The rise in filtered load of sodium, calculated as GFR multiplied by the nearly constant plasma sodium concentration, together with the unchanged or reduced sodium excretion rate shows that the sodium reabsorption rate is increased during acute moderate hyperglycemia. This renal handling of sodium might be due to the interaction between glucose and the sodium transport in the kidney.

In isolated frog kidneys Vogel and Krüger (35) demonstrated that the glucose reabsorption is dependent on the sodium reabsorption in the manner that a high sodium reabsorption rate is accompanied by a high glucose reabsorption rate. In a later work Vogel et al. (36) examined whether the sodium reabsorption was dependent on the glucose reabsorption. It was concluded that this was not the case. However, their results do show that the rate of reabsorption for sodium rose when that for glucose was increased. During the high glucose reabsorption rate the sodium reabsorption rate was on the average 22% higher than during the low glucose reabsorption rate, a significant difference ($p < 0.001$). The fractional reabsorption too was significantly higher.

In consistency with the experimental findings by Bojesen (5) and Leyssac (23), it is probable, according to Kruhoffer (19) that an increase in the proximal water and sodium reabsorption rate may cause an increase in GFR of almost equal quantity. If no other changes take place a slight fall will simultaneously occur in the sodium and water excretion rate. These theoretical considerations together with the results of Vogel et al. (36) may quite well support the view that the increase in GFR during moderate hyperglycemia is caused by the action of glucose on the sodium reabsorption which in turn causes an increase in GFR and a slight fall in the sodium excretion rate.

The slight expansion of the plasma volume and the reduction in the colloid osmotic pressure in plasma found during the induced acute hyperglycemia may be other contributory factors, but, as stated above, the increase in GFR cannot be explained by these changes alone.

In the studies by Mogensen (5) and Ditzel and Junker (12), GFR in young male insulin-treated diabetics was on the average about 25 and 10%

higher than in normals, respectively. The increase in GFR by 10–15% during moderate hyperglycemia demonstrated in the present work supports the contention that an elevated blood glucose concentration is contributory to the increased GFR in diabetics.

ACKNOWLEDGEMENTS

This work was supported by grants from the C. and E. Hertz Foundation, Klog Christian X's Foundation, the Novo Foundation, and the Danish State Research Foundation.

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HUMAN GLOMERULAR BASEMENT MEMBRANE. CHEMICAL COMPOSITION IN DIABETES MELLITUS

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Abstract Glomerular basement membrane (GBM) was isolated from human kidneys obtained at autopsy or nephrectomy in preparation for transplantation. Kidneys from 22 patients were used: 10 patients without history of diabetes mellitus or kidney disease and with normal kidney morphology, 5 with short history of diabetes and minimal pathological changes in the kidneys, and 7 with very advanced diabetic nephropathy. The GBM preparations were individually analysed for amino acids, carbohydrates, phospholipids and cholesterol. The cysteine concentration was found to be lower both in early diabetic nephropathy and advanced diabetic nephropathy than in normal kidneys. In advanced diabetic nephropathy, significant decrease in the content of sialic acid was found. Contrary to other reports there was no increase in the amounts of hydroxylysine or glucosyl-galactosyl-hydroxylysine. The influence of technical factors on the chemical composition of GBM preparations is discussed.

Thickening of the capillary and arteriolar basement membranes is a prominent feature in diabetes mellitus, at least in the advanced stage. These changes in the basement membrane particularly of the glomerular capillaries, have been the subject of extensive investigations (2, 3, 10, 19). A study of the possible biochemical alterations that correlate with this membrane thickening could contribute to our understanding of the pathogenesis of diabetic vascular disease.

Based on our previous experience with the preparation and analysis of normal human glomerular basement membrane (GBM) (26) we have studied the chemical composition of GBM prepared from kidneys with mild or advanced diabetic nephropathy.

MATERIAL AND METHODS

In general only one kidney was available from each patient for this study. Ten normal kidneys were obtained

from persons killed in accidents. Three of the normal kidneys were perfused with 3% low molecular weight dextran containing Edocaine and heparin as used in preparation for kidney transplantation (6), which was not undertaken. Kidneys from 12 diabetic patients were obtained at autopsies performed within 12 h of death or by nephrectomy before kidney transplantation. Glomeruli and human GBM were prepared as previously described from kidneys that were fresh or had been stored at -70°C (26). Samples of fresh kidney tissue were fixed in buffered formalin for light microscopy and sections were studied after staining with hematoxylin-eosin, periodic acid-Schiff and encornine. Snap-frozen tissue was studied by immunofluorescence for the presence of IgG, IgM, fibrin and C_3 as previously described (14). The purity of the GBM preparation was evaluated by phase contrast microscopy in a few cases from each group isolated GBM, after washing in distilled water was fixed in cold 2% glutaraldehyde buffered in sodium cacodylate (pH 7.4) and embedded in Vestopal, and then sections (0.5 μ) were prepared and stained with toluidine blue for light microscopy.

The diagnosis of diabetes mellitus was based upon the clinical picture, including history of chemical diabetes and insulin dependency. The pathological changes in the kidneys were in all cases compatible with diabetic kidney disease: in no case was there reason on clinical or pathological grounds to suspect complicating pyelonephritis or other kidney disease. The glomerular changes observed by light microscopy were evaluated, and the degree of apparent basement membrane thickening, increase in mesangial material and proliferation of endothelial and epithelial cells were semiquantitatively graded from 0 to 3+ with \pm employed for questionable or very localized changes. The number of completely sclerotized glomeruli per 100 glomeruli was counted.

Based on the degree of pathological alterations in the kidneys the material was divided into two groups, early diabetes and advanced diabetes (Table I). Three of the five kidneys in the early diabetic group (nos. 91, 97 and 144) have been discussed in an earlier publication (27) and were then included in the group normal pathology or minimal lesions (Figs. 1-3 ref. 27). All the five patients in this group had shown consistently normal serum creatinine; two had slight proteinuria (nos. 93 and 141). The duration of diabetes was 2-8 years. All diabetic

Table 1 *Semiquantitative evaluation of glomerular pathology*

Kidney no.	Age of pts. (y)	Known duration of diabetes (y)	Basement membrane thickening	Mesangial material increase	Hyalinized glomeruli (%)	Proliferation		Deposition on GBM		
						Mesangial	Endothelial-epithelial	IgG	β C	Fibrin
<i>Early diabetes</i>										
91	18	2	0	0	0	0	0	1+	0	0
92	14	4	0	\pm	0	0	0	1+	0	1
95	32	8	\pm	1+	0	0	0	0	0	0
141	72	8	\pm	2+	0	1+	0	1+	1+	1
144	11	7	\pm	1+	0	\pm	0	1+	1+	1
<i>Advanced diabetes</i>										
124	26	>20	1+	1+	90	0	0	0	0	0
125	42	>20	1+	3+	60	1+	1+	0	0	0
128	35	>20	1	2+	50	1+	0	2+	1+	0
135	27	>20	1+	2+	90	1+	0	0	1+	0
145	32	>20	3	3+	35	0	0	0	2+	0
150	35	>20	3+	3+	30	1+	0	0	2+	0
155	42	>20	1+	1+	30	0	0	1+	1	1+

patients in both groups had been treated with insulin. Only one patient (no. 141) had adult onset type of diabetes mellitus. Five of the seven kidneys in the advanced diabetic group (nos 124, 125, 128, 135 and 150) were included in a previous publication (27). The duration of diabetes in this group was more than 20 years in all cases.

In the normal control group the light microscopy of the glomeruli was considered to be within normal limits in all cases, but slight arteriolar thickening was seen in some of the kidneys from the older patients. The age of the patients in this group was 14–55 years (mean 35). There was no evidence of diabetes in their clinical history.

Chemical analysis for amino acids, carbohydrates, phospholipids, cholesterol and serum proteins, and analysis

for water content, were performed as described previously (26). However for the analysis of glucose, galactose and mannose the Technicon B Automatic Carbohydrate Analyzer was used as described by Catravas (7). Glucosyl-galactosyl-hydroxytyrosine (Glc-Gal-HyLys) and free hydroxytyrosine were measured after alkaline hydrolysis as described by Spiro (23). GBM samples isolated from normal and abnormal kidneys were analysed in random order to increase the reliability of comparisons of group averages.

Statistical significance of group differences was tested using Student's *t*-test. Appropriate formulas for the analysis of independent samples with unequal variances were used when indicated by the *F*-test. Linear regression and correlation coefficients were calculated according to



Fig 1 One of the most severely affected glomeruli in sections from kidney 141. This kidney showed the most abnormal histology in the early diabetic group. A slight to moderate increase in mesangial material is seen PAS stain. $\times 600$.



Fig. 2. A glomerulus from kidney 14 in the advanced diabetic group. About 90% of the glomeruli in this kidney were hyalinized. The remaining glomeruli showed substantial increase in mesangial material and the basement membranes appeared to be thickened. PAS stain. 600.

standard methods (20). A bilateral hypophysis as assessed in all cases. A paired comparison on the basis of age match was not done, as none of the chemical constituents studied showed any age-dependent trend and the age distribution in the three groups of patients was similar.

RESULTS

Glomerular pathology The results of the semi-quantitative evaluation of glomerular pathology are given in Table I. In the early diabetic group no definite thickening of the GBM was apparent by light microscopy. An increase in mesangial material was observed in three of the five kidneys in this group. Typical hyaline mesangial nodules were not observed in any of these kidneys, but were not systematically sought for by serial sectioning. The most advanced changes in glomerular pathology in this group were found in kidney 141 (Fig. 1). Immunofluorescent microscopy showed one kidney only to be completely negative for IgG, β_2 C and fibrin. Linear staining for IgG and fibrin was observed in all glomeruli from four and three kidneys, respectively and granular as well as linear deposition of β_2 C were seen in a few glomeruli from two kidneys.

In kidneys from the advanced diabetic group apparently thickened basement membranes and increased amounts of mesangial material were present in all cases; proliferation was slight or absent (Fig. 2). Immunofluorescent microscopy

A linear deposition of IgG and fibrin was found on the GBM in two and one kidneys, respectively while a linear and granular deposition of β_2 C was found in five kidneys.

Within the early and advanced diabetic groups no correlation was apparent between the degree or type of pathological abnormality seen and the biochemical composition.

Preparation of GBM No special difficulties were encountered in the preparation of GBM from kidneys in the early diabetic group. In the advanced diabetic group a large percentage of the glomeruli remained intact after 7 min of sonication and were removed by the 250 mesh sieve. On phase contrast microscopy or light microscopy of thin sections no difference was observed in purity or appearance between various preparations. The yield of hyphylized GBM was 23.0 ± 7.3 mg, 22.0 ± 6.2 and 36.8 ± 11.5 (mean \pm S.D.) mg per kidney in the normal, early diabetic and advanced diabetic groups, respectively. These differences were not statistically significant.

Water content The overnight weight loss of three GBM samples on drying at 105°C was 4.4, 4.7 and 6.1% respectively. All analytical results in this paper are adjusted for a water content of 51%.

Lipid composition The results of the analyses for phospholipid, phosphorus and cholesterol are given in Table II. One sample only prepared from a kidney in the advanced diabetic group, was

Table II Lipid composition of GBM (mean and range)

No. of samples analysed given in italics

	Normal kidneys	Early diabetes	Advanced diabetes
Phospholipid phosphorus ($\mu\text{g}/\text{mg}$)	0.71 (0.50-0.98) 5	1.02 (0.71-1.48) 5	0.95 (0.70-1.05) 4
Cholesterol ($\mu\text{g}/\text{mg}$)	9.05 (3.50-12.3) 4	9.07 1	21.7 (17.5-30.0) 3
Ratio cholesterol/phospholipid phosphorus	14.3 (8.9-16.4) 4	12.2 1	22.9 (16.2-28.0) 3

cluded from this paper because of a high phospholipid content, as we have previously shown that this reflects contamination. As can be seen in Fig. 3 there was a tendency to an inverse relationship between the values for 4-hydroxyproline and phospholipid phosphorus. Two GBM samples in the early diabetic group prepared from kidneys 91 and 92, were clearly separated from the rest of the material by their high content of phospholipids, 1.48 and 1.34 $\mu\text{g}/\text{mg}$, respectively. These kidneys were the only ones in the material that were obtained from patients dying in diabetic coma; the patients presumably had an abnormally high concentration of lipids in their

blood at the time of death. The GBM cholesterol values were clearly elevated in the advanced diabetic group compared to normal kidneys.

Amino acid composition. The results of the amino acid analysis for the three groups of kidneys are given in Table III in molar concentration and residues per 1000 amino acid residues (T value).

The most distinct difference in the amino acid composition between GBM from normal kidneys and diabetic kidneys was the decrease in cystine content in the diabetic groups (Table IV). The decrease was in molar concentrations significant for the advanced diabetic group ($2p < 0.05$) and almost significant for the early diabetic group ($t = 2.195$ for the molar concentration, $t = 2.291$ for the T-values with $t = 2.365$ and $t = 2.306$ required for $2p < 0.05$). When all normal GBM samples analysed for cystine in this and previous publications (26) ($N = 9$ for molar concentrations, $N = 7$ for T-values) were compared with the combined material of the early diabetic and advanced diabetic groups ($N = 7$) the differences were found to be strongly significant when expressed as molar concentrations ($2p < 0.01$) and significant when expressed as T-values ($2p < 0.05$).

The concentrations of the amino acids considered specific for collagens and basement membranes, 4-hydroxyproline and hydroxylysine, were not significantly increased in the early diabetic and advanced diabetic groups when compared with normal kidneys. The slight increase in the concentration of hydroxylysine was not significant when the combined diabetic groups were compared to normal GBM in this and/or the previous study (26) ($2p > 0.1$). The molar ratio $\text{H}_3\text{Lys}/4\text{-HyPro}$ was not significantly higher in the advanced diabetic group (0.322 ± 0.04) compared to the early diabetic group (0.313 ± 0.041) or to normal kidneys (0.307 ± 0.014). The concentration of proline was slightly decreased in both diabetic

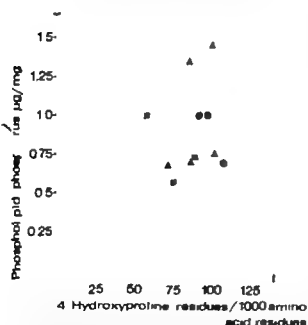


Fig. 3. Relationship between the concentrations of 4-hydroxyproline and phospholipid phosphorus. The values for two samples of GBM from the early diabetic group (A) seem to be separated from the main cluster of values for GBM from the normal (○), early diabetic and advanced diabetic (●) groups. The linear regression coefficients for the main cluster and for the normal GBM values are not significantly different from 0.

Table III. Amino acid composition of human GBM

	Amount ($\mu\text{M}/100 \text{ ng}$, mean \pm S.E.M.)			Residues per 1 000 amino acid residues (T values)		
	Normal kidney (N=8)	Early diabetes (N=4)	Advanced diabetes (N=5)	Normal kidneys (N=10)	Early diabetes (N=5)	Advanced diabetes (N=5)
3-hydroxyproline	14.1 \pm 0.72	14.7 \pm 0.90	12.1 \pm 1.37	18.5 \pm 0.93	18.6 \pm 1.62	16.8 \pm 1.95
4-hydroxyproline	11.1 \pm 4.35	68.6 \pm 3.29	62.3 \pm 4.99	82.2 \pm 4.52	88.6 \pm 5.43	88.1 \pm 7.92
Aspartic acid	50.6 \pm 1.57	48.4 \pm 1.74	45.5 \pm 2.78	67.6 \pm 1.39	66.3 \pm 1.65	63.7 \pm 2.59
Threonine	28.1 \pm 0.82	27.9 \pm 1.17	27.7 \pm 1.62	37.6 \pm 0.56	37.5 \pm 0.97	38.9 \pm 2.05
Serine	37.1 \pm 1.59	35.8 \pm 1.17	31.6 \pm 2.11	49.5 \pm 1.07	48.4 \pm 0.96	45.7 \pm 1.53
Glutamic acid	71.2 \pm 2.37	68.4 \pm 1.49	65.8 \pm 2.88	93.9 \pm 2.13	91.0 \pm 1.32	92.4 \pm 1.93
Proline	46.8 \pm 2.10	41.8 \pm 1.74	42.6 \pm 3.25	62.5 \pm 1.67	58.4 \pm 2.69	59.5 \pm 3.18
Glycine	199.0 \pm 5.01	152.7 \pm 3.89	156.3 \pm 7.97	210.5 \pm 4.14	205.5 \pm 7.85	220.1 \pm 7.62
Alanine	41.0 \pm 1.23	39.0 \pm 0.74	37.2 \pm 2.17	54.8 \pm 0.66	52.1 \pm 0.99*	52.2 \pm 1.83
Valine	25.8 \pm 1.01	26.4 \pm 1.62	26.7 \pm 0.96	34.8 \pm 1.10	35.7 \pm 1.46	37.7 \pm 2.17
Methionine	12.2 \pm 0.72	11.2 \pm 1.04	12.4 \pm 1.39	17.6 \pm 1.08	16.4 \pm 1.64	18.1 \pm 2.00
Isolecucine	23.4 \pm 0.58	22.9 \pm 0.33	23.2 \pm 0.31	31.7 \pm 0.64	31.8 \pm 0.79	32.6 \pm 0.72
Leucine	49.8 \pm 1.34	48.2 \pm 1.29	45.1 \pm 2.14	66.8 \pm 1.18	63.8 \pm 1.04	63.2 \pm 1.74
Tyrosine	11.6 \pm 0.44	11.6 \pm 0.46	12.2 \pm 1.17	16.0 \pm 0.47	16.0 \pm 0.44	16.9 \pm 1.19
Phenylalanine	21.8 \pm 0.35	23.4 \pm 1.11	21.7 \pm 0.86	29.1 \pm 0.43	31.6 \pm 0.94	30.5 \pm 0.78
Hydroxytyrosine	18.5 \pm 0.91	19.1 \pm 1.70	19.5 \pm 1.39	24.9 \pm 0.97	27.0 \pm 2.17	27.3 \pm 1.65
Lysine	14.9 \pm 0.85	18.4 \pm 1.60*	15.9 \pm 1.88	20.4 \pm 0.99	24.4 \pm 1.65	22.2 \pm 2.02
Histidine	10.8 \pm 0.52	12.3 \pm 0.67	10.1 \pm 1.21	14.5 \pm 0.61	16.6 \pm 0.63	14.0 \pm 1.27
Arginine	30.5 \pm 1.19	27.1 \pm 4.45	30.3 \pm 3.09	41.5 \pm 1.64	38.1 \pm 4.74	42.2 \pm 3.07
Half-cystine	18.3 \pm 0.62	13.7 \pm 1.99	13.5 \pm 2.09*	26.1 \pm 1.63	18.9 \pm 2.68	19.6 \pm 3.03

Average \pm S.E.M. Nine, five and four samples, respectively were analysed in triplicates or duplicates and the averages were used for calculation of S.E.M. Six, four and three samples, respectively were analysed in triplicates for cystine and methionine. Residues per 1 000 amino acid residues calculated without regard to tryptophan, which was not analysed. Because of low mean of molar concentrations of asialic acids, 2 samples of normal GBM and 1 sample in the early diabetic group were used only for the calculation of residues per 1 000 amino acid residues.

* $0.05 < 2p < 0.10$, $0.02 < 2p < 0.05$ when compared to normal GBM.

groups as compared to normal kidneys. There was a slight increase in lysine and histidine ($2p < 0.10$ and $2p < 0.05$ respectively) in the early diabetic but not in the advanced diabetic group. A small decrease in the concentration of alanine and a small increase in the concentration of phenylalanine was evident for both diabetic groups when

compared to the normal kidneys, but the differences reached significance ($2p < 0.05$) only when the early and advanced diabetic groups were combined and the values were expressed as residues per 1 000 amino acid residues. The concentration of serine was also slightly decreased in the diabetic kidneys.

Table IV. Comparison of data for cystine in normal and diabetic kidney GBM

	Concentration of half-cystine	
	Amount ($\mu\text{M}/100 \text{ ng}$)	Residues per 1 000 amino acid residues (T)
I. Normal kidneys	18.3 \pm 0.62 (N=5)	26.1 \pm 1.63 (N=6)
II. Early diabetes	13.7 \pm 1.99 (N=4)	18.9 \pm 2.68 (N=4)
	$2p < 0.10$ (I versus II)	$2p < 0.10$ (I versus II)
III. Advanced diabetes	13.5 \pm 2.09 (N=3)	19.6 \pm 3.03 (N=3)
	$2p > 0.05$ (I versus III)	$2p > 0.10$ (I versus III)
IV. Normal kidneys (present and previous publications (26))	17.9 \pm 0.47 (N=9)	25.2 \pm 1.62 (N=7)
II III. All diabetic kidneys	13.6 \pm 1.33 (N=7)	19.2 \pm 1.84 (N=7)
	$2p < 0.025$ (I versus II+III)	$2p < 0.002$ (I versus II+III)
	$2p > 0.01$ (IV versus II+III)	$2p < 0.05$ (IV versus II+III)

Table V Carbohydrate composition of human GBM ($\mu\text{g}/\text{mg}$ mean \pm S.E.M.)

No. of samples analysed in italics. All samples in this Table analysed in duplicates

	Normal kidney	Early diabetes	Advanced diabetes	Early and advanced diabetes
Sialic acids ^a	7.8 \pm 0.17 9	6.8 \pm 0.57 5	5.6 \pm 0.60 6	6.3 \pm 0.43 11
Glucosamine ^b	16.2 \pm 0.43 6	20.2 \pm 3.08 5	15.3 \pm 1.16 5	17.8 \pm 1.44 10
Galactosamine ^c	0.87 (0-2.0) 6	0.89 (0-1.3) 5	0.10 (0-0.5) 5	0.45 (0-1.3) 10
Glucose	23.0 \pm 0.26 5	77.2 \pm 1.15 5 **	26.5 \pm 1.68 5	26.9 \pm 0.97 10*
Galactose	29.6 \pm 0.28 5	28.2 \pm 1.84 5	27.2 \pm 2.24 5	27.8 \pm 1.35 10
Mannose	6.1 \pm 0.39 5	6.3 \pm 0.53 5	5.7 \pm 0.35 5	6.1 \pm 0.33 10
Fucose	1.45 \pm 0.086 5	1.55 1	1.46 \pm 0.039 4	1.50 \pm 0.045 5

^a Calculated as N-acetylneuraminic acid.^b Calculated as N-acetylglucosamine.^c Calculated as N-acetylgalactosamine.

0.01 < 2p < 0.02, 2p < 0.01 when compared to normal GBM.

Glucosyl galactosyl-hydroxylysine When purified Glc-Gal HyLys and free hydroxylysine were subjected to alkaline hydrolysis and analysed on the amino acid analyser the degree of recovery was found to be the same for both. The average difference between duplicates, when 13 duplicate samples were hydrolysed and analysed, was found to be 4.9% of the higher value. Glc-Gal HyLys was calculated as percentage of total hydroxylysine (Glc-Gal-HyLys plus unsubstituted hydroxylysine) and was found to be 72.3 ± 1.53 (mean \pm S.E.M. for ten GBM samples, three analysed in duplicates) for the normal kidneys. For the early diabetic group the percentage was 73.5 ± 1.86 (five GBM samples, two analysed in duplicates) and for the advanced diabetic group 72.6 ± 2.07 (six GBM samples, two analysed in duplicates). These values were not significantly different.

Carbohydrate composition. The results of the analyses for carbohydrates are summarized in Table V. There was a decrease in the amount of N-acetylneuraminic acid present in the diabetic kidney GBM samples, and this difference between the advanced diabetic group and the normal kidneys is strongly significant (*p < 0.02). Also the difference between the mean values for normal GBM and the combined early and advanced diabetic group $6.3 \pm 0.43 \mu\text{g}/\text{mg}$, is strongly significant (2p < 0.005).

The glucose concentration was significantly elevated in the early diabetic and advanced diabetic groups compared to normal GBM. Only five normal GBM samples were analysed in this series. When values for the eight normal GBM preparations reported previously were combined

with the data in the present study no significant difference between normal and diabetic GBM was seen.

DISCUSSION

It is impossible to obtain an absolutely pure GBM preparation by any currently used method. As indicated in our previous paper (26) the original procedure of Krakower and Greenspan (11) results in GBM preparations with low content of hydroxyproline and high content of phospholipids, and using phase microscopy the preparations seem to be rather heavily contaminated with a debris of probably cellular origin. The modification described by Spiro (71) results in preparations of greater purity as judged by the microscopic appearance and by the higher hydroxyproline and lower phospholipid content. Removal of glomeruli resistant to sonication as proposed by us (76) is of great importance when GBM from normal and diseased kidneys are compared. For such a comparison a chemical index of purity such as the phospholipid content, is also valuable provided that normal and diabetic pure GBM contain no phospholipid or similar concentrations of phospholipids. No method has been found to evaluate the possible contamination by mesangial material in the GBM preparations.

An increase was seen in the cholesterol concentrations in GBM of the advanced diabetic group although only three samples were analysed in that group. The importance of this observation, not previously described, is not clear.

The most striking difference in the chemical

composition between GBM samples from normal and diabetic kidneys was the decrease in cystine content in diabetic GBM. This decrease was seen in both early and advanced diabetes. When cystine values derived from all the diabetic GBM samples were compared with values for all the normal human GBM samples analysed by us, the decrease was statistically strongly significant.

This decrease in content of one amino acid cannot be explained on the basis of contamination of the samples with cellular material, as this would have reduced to an equal extent the concentration of e.g., 3- and 4-hydroxyproline, hydroxylysine and glycine, a pattern which we have repeatedly seen in GBM samples obtained by centrifugation at a higher g-force than used in this study. Contamination with collagen can also be excluded as the cause of this change in composition, as this would increase the concentrations of proline, glycine and alanine and decrease the concentrations of threonine, methionine, isoleucine, tyrosine and hydroxylysine, changes that were not seen in these samples. A contaminating material that could explain the decrease in cystine would thus have to be of a composition very similar to GBM but with a low cystine content. Whether the mesangial intercellular substance fulfils these criteria cannot be decided at this time as no satisfactory method has been described to obtain pure mesangial material.

By light microscopy no increase in mesangial material could be found in two kidneys in the early diabetes group (nos. 91 and 92). The GBM prepared from these kidneys had a low cystine content, similar to the mean values discussed.

The biological importance of a decrease in the cystine concentration in diabetic GBM is not clear but a decrease in the number of disulfide bridges, whether inter- or intramolecular could well be a cause of increased permeability to larger molecules, such as serum albumin.

For the remaining amino acids some dissimilarities were observed between the groups, but these differences were quite small and of low statistical significance. When so many numbers are compared, some differences of small statistical significance should occur according to the laws of probability. No major differences in the concentrations of hydroxytyrosine, lysine or hydroxyproline were found.

In the carbohydrate analysis a strongly significant

decrease in the sialic acid concentration was observed in GBM prepared from kidneys with advanced diabetic nephropathy. GBM from the group early diabetes also showed a slight but not significant decrease in the sialic acid content. No difference was found in the concentrations of hexosamines and fucose, indicating that the number of heteropolysaccharide molecules was unchanged (22). The most probable explanation is that the amount of sialic acid per heteropolysaccharide molecule in the GBM is decreased in the advanced diabetic group. This question can only be answered by a careful study of the composition of the isolated heteropolysaccharide in GBM samples prepared from a large number of kidneys showing diabetic nephropathy.

The amount of glucose was decreased and the galactose/glucose ratio increased in the five normal GBM samples analysed in this study compared to the eight normal GBM samples reported in our previous paper (26). The amount of glucose in the two diabetic GBM groups in the present study was similar to that reported for normal GBM in our previous publication. Thus, our studies do not conclusively prove the presence or absence of a change in diabetic GBM in the concentration of glucose and galactose, but minor differences may have been overlooked because of the relatively large intersample variation for glucose and galactose.

Törnblom (25) described a method for isolating the hyalinized glomeruli resistant to ultrasonic vibration from diabetic kidneys and compared the biochemical composition of this hyalinized material to that of intact glomeruli isolated from normal human kidneys. No consistent difference was seen between the preparations obtained from normal and from diabetic kidneys (18).

In their extensive study on GBM from normal and diabetic human kidneys Lazarow and Speidel (12) found no difference in the overall chemical composition, except for a slight increase in the ratio hydroxyproline/nitrogen in the diabetic GBM. The use of alkali in the preparation of GBM in their study may have led to alterations in the GBM glycoprotein.

Behringer and Spuro (1) reported data on the chemical composition of GBM isolated from kidneys obtained at autopsy from eight diabetic individuals showing moderate to severe degrees of mesangial and basement membrane changes.

They found in the diabetic GBM samples a significant increase in the hydroxylysine content and a decrease in the lysine content, while the sum of the concentrations of hydroxylysine and lysine was constant. The amount of Glc-Gal-H₃Lys was also increased in GBM from the diabetic kidneys.

We cannot confirm the observations of Beisswenger and Spiro (1) of an increased hydroxylation of lysine in GBM from diabetic kidneys. On the contrary we found a slight and statistically not significant increase ($0.1 < 2p < 0.2$) in the lysine concentration in GBM from the early diabetic group. In our experience a high value for lysine in GBM isolated from normal kidney is found in samples contaminated with cellular material. A high lysine value is often found together with a high phospholipid concentration but in our limited material this correlation did not quite reach statistical significance. It is conceivable that under certain circumstances the thicker basement membrane from diabetic kidneys may give a GBM preparation of higher purity than GBM from normal kidneys. The lysine value reported by Beisswenger and Spiro for GBM from normal kidneys (25.4 ± 0.94 residues per 1000 amino acid residues) is higher than ours (20.4 ± 0.99). Differences in the purity of the GBM preparations obtained could possibly explain the discrepancy between the results obtained in our study and those reported by Beisswenger and Spiro.

The source of the diabetic kidneys could also be of importance. All the kidneys in the advanced diabetic group in this study were obtained by nephrectomy from patients awaiting kidney transplantation. These patients had been hospitalized for some time before the operation and their diabetes was well controlled. In the early diabetic group only two patients are known not to have been in good diabetic control when the kidneys were obtained. The kidneys used by Beisswenger and Spiro were obtained at autopsy. It is possible that insufficient metabolic control during the final period before death in these patients may have influenced the composition of the GBM, as is indicated by the sensitivity of the kidney glucosyl transferase to insulin or glucose concentrations in the experiments on alloxan diabetic rats described by Spiro and Spiro (24).

A striking difference in the carbohydrate composition in this study between GBM isolated from normal and diabetic kidneys is a decreased con-

centration of sialic acid in the diabetic preparations. The presence of sialic acid in the basement membrane proper has been challenged primarily on histochemical grounds. Mobos and Skoza (15) and Michael et al. (13) found that the basement membrane shows no or only a weak reaction with colloidal iron. Nolte and Ohkuma (17) Jones (9) and Geyer et al. (8) on the other hand, found a weak staining for colloidal iron in the laminae rara externa and interna. The basement membrane may be a not entirely homogeneous structure and variations in the proportions of laminae rara and laminae densa could possibly explain the difference in sialic acid content between normal and diabetic GBM. Mohos and Skoza (16) pointed out that the sialic acid concentration decreased with decreasing centrifugal force when acidified glomeruli were centrifuged in 1.5 M NaCl, indicating that at least some of the sialic acid found in the basement membrane preparations could be contained in cellular material contaminating the preparation.

It has recently been shown by Blau and Michael (5) that in rats, made nephrotic with puromycin aminonucleoside, the sialic acid concentrations are decreased in GBM and glomerular cell membrane preparations. Blau and Haas (4) have also shown that the colloidal iron staining for sialic acid residues in the glomeruli is decreased or absent in children with proteinuria secondary to glomerular disease, including the minimal lesion nephrotic syndrome. Whether the decreased staining for sialic acids is secondary to the proteinuria or related to the cause of proteinuria is not clear.

No histochemical study of the basement membrane in diabetic kidneys using specific stains for polyanions has been published, but could be of value to explain our results.

Despite its thickness, the GBM in kidneys from patients with diabetes mellitus appears to have an increased permeability to serum proteins. The finding in this study of decreased concentrations of sialic acids and cystine may be related to this phenomenon. Further studies, including both human kidneys and kidneys from animals with experimental or spontaneous diabetes, using histochemical and biochemical methods, will obviously be necessary.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Diabetes Association and the National Institutes of Health (AM 13756, AM 12373, HE 05662, HE 06134, H 1263C).

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HUMAN GLOMERULAR BASEMENT MEMBRANE. CHEMICAL COMPOSITION IN GLOMERULONEPHRITIS AND PYELONEPHRITIS

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Abstract. The chemical composition of glomerular basement membrane (GBM), isolated from 18 kidneys in advanced stage of renal disease, has been studied. On the basis of morphological changes and clinical observations 7 kidneys from 7 patients were classified as showing chronic pyelonephritis, including interstitial nephritis, 5 as chronic membranoproliferative glomerulonephritis, 2 as idiopathic membranous glomerulonephritis, and 4 as chronic glomerulonephritis of unspecified type. Ten normal kidneys were included as controls. For several constituents obvious differences were found between GBM isolated from diseased and normal kidneys, most pronounced for the chronic membranoproliferative glomerulonephritis and idiopathic membranous glomerulonephritis groups. The differences could not be explained solely by contamination of the diseased kidney GBM with collagen. More than one simultaneous process may have contributed to the observed changes in the biochemical composition of diseased GBM.

Early in the course of chronic glomerulonephritis and in the end stage of almost every other kidney disease morphological changes are seen in the glomerular basement membrane (GBM) particularly as diffuse or localized thickening. Increased glomerular capillary permeability to macromolecules is a regular feature in glomerulonephritis. Several investigations have shown that the GBM is an important determinant of glomerular capillary permeability at least for the larger serum proteins. Knowledge of the biochemical correlates of these morphological and functional alterations could contribute to our understanding of the nature and pathogenesis of glomerular disease. As a first step in this direction a study has been undertaken of the chemical composition of GBM isolated from kidneys showing pathological changes characteristic of certain types of glomerulonephritis or pyelonephritis.

MATERIAL AND METHODS

The ten normal kidneys were the same as described in previous publication (17). Diseased kidneys were removed through nephrectomy either as preparation for kidney transplantation, or in one case (kidney 133) because of psoas-arteric obstruction with intrapelvic stones. In general only one kidney from each patient was available for this study. The methods for isolating glomeruli and preparing GBM and for the pathological and immunopathological work have been described in our previous publications (15, 16, 17).

The diseased kidneys used in this investigation were generally sclerotic, and in particular the severely scarred pyelonephritic kidneys were difficult to dissect and to pass through the 100-mesh steel sieve. In preliminary experiments it was found that if greater pressure was used in forcing renal cortex through the sieve, there was an increase in the amount of contamination with tubular elements. For this reason the protocol for preparation of glomeruli and GBM from diseased kidneys was the same as that used for normal kidneys.

The preparations of glomeruli obtained from diseased kidneys appeared by phase contrast microscopy to be equally as pure as those from normal kidneys. No free tubular fragments were seen, but some small tubular segments remained attached to few glomeruli and could not be removed. After 7 min of sonication a considerable number of hyaline glomeruli remained apparently intact and were removed on the fine mesh sieve. Such sonication resistant glomeruli were washed four times with distilled water and lyophilized.

Light microscopy

Sections stained with hematoxylin-eosin and periodic acid-Schiff were studied. The number of hyalinized glomeruli per 100 glomeruli was counted. The morphological and immunofluorescence observations are recorded in Table I.

The diagnosis of chronic membranoproliferative glomerulonephritis (CMPGN), five cases, was made on the basis of criteria described in previous publications (8, 11). Increased size and lobulation of glomeruli, pronounced mesangial proliferation and thickened basement membranes were seen in all kidneys (Fig. 1). Three of the pe-

Table I. *Semiquantitative evaluation of glomerular pathology*

Kidney no.	Age of pts. (y)	Basement membrane thickening	Mesangial material increase	Proliferation		Hyalinized glomeruli (%)	Deposition on GBM		
				Mesangial	Endothelial-epithelial		IgG	β_2 C	Fibrin
<i>CGA</i>									
113	44	1	2	1+	1+	25	0	0	—
131	24	3	1+	2+	2+	60	0	0	0
134	22	1	2+	1	1+	75	+	1+	0
151	22	3	3	2+	3+	60	0	2+	0
<i>CAIPGN</i>									
97	16	3	3	3+	3	40	2+	0	0
136	19	3+	3	3+	3+	30	1+	3+	1
138	17	3	3+	3+	3+	25	1+	1+	1
146	10	3	3	3+	1+	20	1+	2+	0
147	16	3+	3+	3	1+	20	1	3+	0
<i>IMGN</i>									
129	32	3	1+	1	±	80	2+	2+	—
149	39	3	1	1+	±	40	3+	1	—
<i>CPN</i>									
117	22	0	3	3+	3+	60	—	—	—
118	28	0	1+	1+	1+	80	0	1+	0
127	13	1+	1	1	1	20	0	0	0
133	60	0	1	1+	1+	5	0	0	—
140	34	0	2+	3+	3+	45	0	1+	0
148	13	0	1+	0	0	5	0	1+	0

kidneys from whom kidneys were taken (kidneys 138, 146 and 147) had shown low values for β_2 C/ β_2 A in several serum samples obtained during the course of their illness. In the two remaining cases serum values for β_2 C/ β_2 A were measured only when their kidney disease had progressed to uraemia, and were then normal. A characteristic lobular glomerular deposition of β_2 C/ β_2 A and properdin was seen by immunofluorescence in all kidneys in this group, except kidney 97 where only a very focal and non-characteristic staining for these proteins was found. Nega-

tive immunofluorescence in CAIPGN has previously been described as an end stage phenomenon (6).

In the two cases of idiopathic membranous glomerulopathy (IMGN) the pathological changes in the glomeruli were characteristic, with extremely thickened GBM and with minimal degree of mesangial proliferation (Fig. 2). The finding of large "saw-tooth" deposits of IgG and β_2 C/ β_2 A by immunofluorescence microscopy confirmed the diagnosis.

The kidneys in the group chronic glomerulonephritis



Fig. 1 High power view of part of glomerulus from kidney 97 (CAIPGN). The sclerotic area of the mesangial area, that extends into the periphery of the loop and occludes the capillary lumen, is seen. The basement membrane is duplicated and cells are interpositioned between the layers. PAS stain. $\times 800$.



Fig. 2 Part of glomerulus from kidney 129 showing the characteristic features of chronic IMGN with thick and stiff membranes and absence of proliferative changes. PAS stain. $\times 600$.



Fig 3 Glomerulus from kidney 113 (CGN). While many glomeruli in this kidney were completely sclerosed, others showed only moderately advanced changes. Increased mesangial material and periglomerular fibrosis were found. PAS stain. 250.

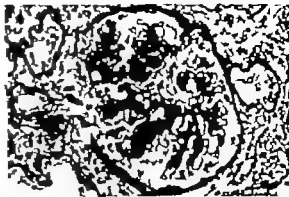


Fig 4 Sections from kidneys in the CPN group showed many hyalinized glomeruli. In the remaining glomeruli there was little evidence of thickening of the basement membranes, but an advanced mesangial sclerosis was common. This section is from kidney 117. PAS stain. 400.

(CGN), four cases, showed advanced glomerular pathology with variable degrees of basement membrane thickening, increase of mesangial material and proliferative changes (Fig. 3). The pathological features characteristic of CNMPGN and IMGN are not observed. No history of urinary tract infection was obtained.

The diagnosis of chronic pyelonephritis (CPN) was based on the clinical history with repeated and protracted periods of urinary tract infection with fever and on the macroscopic appearance of the kidney. The kidneys from five of the six cases in this group were generally very shrunken and showed large scars from the medullary area to the cortex; round cell infiltration was prominent. Some glomeruli were of almost normal appearance, but most were either completely hyalinized or showed hyalinization of parts of the glomerulus (Fig. 4). The GBM did not appear thickened except in kidney 127 where the thickening was very slight. Kidney 140 was different from the other kidneys of this group. The patient from whom this kidney was taken had only experienced few short bouts of urinary tract infections, and the kidney showed no evidence of scarring. Light microscopy showed severe interstitial fibrosis, but little round cell infiltration. The diagnosis chronic interstitial nephritis is probably more correct than CPN in this case. The glomerular changes were, however, very similar to those in the CPN group. A number of glomeruli showed little pathological change and the GBM was not thickened. This kidney was included in the CPN group.

Chemical analyses

The methods for analysing amino acids, phospholipids, cholesterol and sialic acid have been described previously (15). Glucosyl-galactosyl-hydroxylamine (Glc-Gal-Hyl)₂s was measured after alkaline hydrolysis according to Apéro (14). Glucose, galactose and mannose were analysed on the Technicon® Carbohydrate Analyser as described by Catreva (4). The same factor for correction for water content was used as described previously (17).

Statistical methods

The statistical methods employed have been described previously (17).

RESULTS

Preparation of GBM

The GBM prepared from diseased kidneys was no more contaminated with cellular debris than normal GBM as judged by phase contrast microscopy and light microscopy of stained thin sections. The average yield of lyophilized GBM in the four groups was (mg/whole kidney): CNMPGN 15.9 (10–14), MGN 12.5 (10–15), CGN 12.8 (7–15) and CPN 8.9 (5–16). The yield was lower from the pyelonephritic (8.9 ± 1.4) than from the normal kidneys (23.0 ± 7.3) (mean \pm S.E.M.). The difference was statistically not significant ($0.05 < 2p < 0.10$).

Chemical composition

Lipid composition. Because of the low yield of GBM from the diseased kidneys, and in particular the pyelonephritic kidneys, insufficient material was available to carry out all of the desired chemical analyses on each individual sample. The largest amount of material was required for the analyses for phospholipids and cholesterol, 3–5 and 2–4 mg of lyophilized GBM respectively and these analyses could thus be performed on only a small number of samples of GBM from diseased kidneys. The results are given in Table II. In general the amounts of phospholipids and pos-

Table II Lipid composition of GBM (mean and range)

No. of GBM samples analysed given in *italics*

	Normal kidney	CMPGN	IMGN	CGN
Phospholipid phosphorus ($\mu\text{g}/\text{mg}$)	0.71 (0.50-0.98) 5	1.02 (0.96-1.29) 5	1.05 1	0.94 (0.90-0.98) 2
Cholesterol ($\mu\text{g}/\text{mg}$)	9.05 (5.50-12.3) 4	13.8 (12.8-15.0) 3	—	—
Ratio cholesterol/phospholipid phosphorus	14.3 (8.9-16.4) 4	12.7 (9.9-14.9) 3	—	—

Table III Amino acid composition of GBM isolated from normal and diseased human kidneys (concentration in $\mu\text{M}/100\text{ mg}$)

	Normal kidneys (N=8)	CGN (N=4)	CMPGN (N=5)	IMGN (N=2)	CPN (N=6)
3-hydroxyproline	14.1 \pm 0.72 (10.2-17.4)	11.6 (8.3-12.9)	6.1 \pm 0.82 (3.6-8.5)	9.3 (7.0-11.6)	8.7 \pm 0.56 (7.1-11.2)
4-hydroxyproline	63.1 \pm 4.35 (41.3-77.4)	61.6 (49.6-68.2)	33.8 \pm 2.92 (25.9-42.4)	43.1 (30.1-56.1)	48.2 \pm 3.52 ^a (40.0-62.6)
Aspartic acid	50.6 \pm 1.57 (44.1-57.9)	47.7 (43.7-49.9)	39.4 \pm 3.38 (48.4-69.8)	55.5 (45.4-63.6)	52.6 \pm 1.96 ^a (48.2-58.7)
Threonine	28.1 \pm 0.82 (25.5-31.5)	4.9 (24.3-25.9)	34.9 \pm 1.94 (28.4-38.2)	32.1 (23.1-41.1)	28.4 \pm 1.58 (22.8-33.0)
Serine	37.1 \pm 1.59 (31.3-45.7)	33.1 (30.6-35.3)	43.1 \pm 3.11 (32.2-48.5)	38.9 (29.5-48.3)	36.7 \pm 1.33 (32.2-41.4)
Glutamic acid	71.2 \pm 2.57 (61.5-82.8)	65.6 (62.9-70.4)	80.9 \pm 5.42 (65.7-94.9)	74.3 (57.4-91.1)	70.3 \pm 3.05 (62.3-77.9)
Proline	46.8 \pm 2.1 (39.5 \pm 55.2)	55.6 (43.0-62.0)	49.3 \pm 1.56 (46.9-51.3)	47.6 (35.3-59.9)	46.8 \pm 1.41 (43.2-55.0)
Glycine	159.0 \pm 5.01 (132.2-180.9)	178.9 (124.0-213.4)	117.9 \pm 7.04 (97.7-135.9)	137.2 (99.2-175.0)	139.0 \pm 5.97 ^a (125.5-160.5)
Alanine	41.0 \pm 1.23 (36.7-46.6)	49.1 (43.5-59.2)	48.0 \pm 3.39 (37.1-57.7)	45.3 (35.1-55.5)	46.9 \pm 2.44 (36.6-53.7)
Valine	25.8 \pm 1.01 (19.8-29.0)	25.1 (22.8-27.7)	32.3 \pm 2.33 (26.1-38.3)	33.8 (30.2-37.4)	27.9 \pm 1.66 (21.7-34.2)
Methionine	12.2 \pm 0.72 (11.4-15.3) (N=5)	12.4 (N=1)	15.6 (14.6-16.6) (N=3)	10.2 (N=1)	9.4 (N=1)
Isoleucine	23.4 \pm 0.58 (20.4-25.6)	21.2 (20.6-21.8)	25.3 \pm 1.33 (22.3-29.8)	29.3 (27.0-31.6)	22.3 \pm 1.61 (15.7-27.3)
Leucine	49.8 \pm 1.34 (46.2-55.6)	42.7 (41.5-43.8)	54.7 \pm 4.00 (43.7-65.7)	53.7 (40.8-66.6)	47.7 \pm 2.85 (37.2-56.1)
Tyrosine	11.6 \pm 0.44 (9.8-13.2)	9.5 (7.9-11.7)	18.8 \pm 0.82 (16.8-21.2)	16.1 (10.4-21.7)	10.9 \pm 0.75 (8.6-13.3)
Phenylalanine	21.8 \pm 0.55 (19.5-23.3)	19.4 (16.3-21.1)	26.5 \pm 1.49 (21.7-30.4)	25.3 (18.1-32.5)	21.1 \pm 1.73 (16.2-28.0)
Hydroxylysine	18.5 \pm 0.91 (13.1-22.0)	18.0 (16.1-19.9)	12.0 \pm 0.33 (10.9-13.3)	15.7 (11.8-19.6)	14.8 \pm 0.93 (1.0-17.5)
Lysine	14.9 \pm 0.85 (11.5-18.5)	16.6 (14.7-19.1)	25.7 \pm 1.49 (20.5-28.8)	20.4 (18.7-22.0)	20.2 \pm 2.11 (15.6-28.1)
Histidine	10.8 \pm 0.52 (8.3-14.3)	9.9 (8.6-12.2)	12.63 \pm 0.88 (9.2-14.2)	11.6 (9.8-13.4)	10.8 \pm 0.83 (8.5-13.5)
Arginine	30.5 \pm 1.19 (25.8-36.6)	31.8 (30.4-33.8)	37.7 \pm 2.82 (27.7-43.7)	33.0 (29.2-36.9)	31.0 \pm 2.17 (23.0-38.5)
Half-cystine	18.3 \pm 0.62 (15.9-19.5) (N=5)	12.2 (N=1)	19.5 (17.2-22.5) (N=3)	17.1 (N=1)	14.5 (N=1)

Average \pm S.E.M. (because of slightly low molar sums, in 2 samples, possibly because of contamination with sodium chloride, 8 samples only were included). Duplicate or triplicate analyses are carried out on 9, 2, 5, 2 and 4 samples, respectively, at the different groups. The average values were used for the calculation of S.E.M. The analyses for cystine and methionine were performed in triplicates.

0.05 < 2p < 0.10, 0.02 2p < 0.05, 0.01 2p < 0.02, 2p < 0.01 when compared to normal GBM.

Table IV Amino acid composition of GBM isolated from normal and diseased human kidneys. Residues per 1 000 amino acid residues (mean \pm S.E.M. and/or range)

	Normal kidney (N=10)	CGN (N=4)	CMPGN (N=5)	IMGN (N=2)	CPN (N=6)
3-hydroxyproline	18.5 \pm 0.93 (14.3–24.4)	15.0 (12.5–16.0)	8.0 \pm 0.93 (4.9–10.1)	12.3 (11.8–12.8)	12.4 \pm 0.78 (10.0–15.5)
4-hydroxyproline	82.2 \pm 4.52 (57.7–103.4)	80.2 (74.6–85.5)	41.6 \pm 2.66 (35.9–49.9)	56.4 (51.6–61.2)	68.3 \pm 4.56 (56.2–84.7)
Aspartic acid	67.6 \pm 1.39 (61.9–75.6)	62.7 (57.1–73.6)	78.6 \pm 2.06 (73.3–82.7)	74.7 (71.8–77.5)	74.4 \pm 2.07** (66.9–81.2)
Threonine	37.6 \pm 0.56 (35.8–41.9)	32.7 (30.4–37.3)	46.2 \pm 1.57 (43.3–52.1)	42.3 (39.6–45.0)	38.5 \pm 1.68 (32.9–42.3)
Serine	49.5 \pm 1.07 (43.8–55.0)	43.4 (38.3–49.4)	56.9 \pm 2.85 (49.1–65.2)	51.6 (30.5–52.7)	51.6 \pm 1.19 (48.5–56.9)
Glutamic acid	93.7 \pm 2.13 (83.5–105.6)	84.0 (78.9–94.6)	106.8 \pm 2.19 (100.3–112.1)	98.9 (96.3–99.7)	99.3 \pm 2.23 (93.5–107.8)
Proline	62.5 \pm 1.67 (55.5–69.6)	72.3 (67.4–77.2)	63.9 \pm 4.02 (58.8–81.4)	63.8 (60.3–65.6)	66.3 \pm 2.78 (55.4–70.5)
Glycine	210.5 \pm 4.14 (184.9–231.5)	231.7 (186.5–267.8)	156.3 \pm 5.61 (135.6–169.4)	180.5 (164.7–192.3)	197.1 \pm 8.97 (173.3–224.8)
Alanine	54.8 \pm 0.66 (51.9–58.1)	64.1 (54.2–73.8)	63.2 \pm 1.82 (56.6–67.2)	60.3 (60.0–60.7)	66.3 \pm 3.06** (57.0–75.8)
Valine	34.8 \pm 1.10 (27.4–60.5)	33.2 (28.6–41.7)	42.7 \pm 1.61 (38.5–46.8)	46.2 (40.8–51.7)	39.3 \pm 1.54** (32.8–43.7)
Methionine	17.6 \pm 1.08 (N=6) (13.8–21.4)	16.3 (N=1)	20.0 (N=3) (18.8–21.7)	11.4 (N=1)	11.7 (N=1)
Isoleucine	31.7 \pm 0.84 (28.3–34.9)	27.8 (26.7–30.9)	33.4 \pm 0.46 (32.0–34.7)	40.4 (34.5–46.3)	31.4 \pm 1.85 (23.6–35.3)
Leucine	66.8 \pm 1.18 (62.5–73.5)	56.1 (51.9–64.9)	72.1 \pm 2.47 (63.4–74.2)	71.3 (69.9–72.1)	67.3 \pm 3.11 (56.1–78.9)
Tyrosine	16.0 \pm 0.47 (13.7–18.0)	12.6 (10.3–14.7)	24.9 \pm 0.67 (23.3–26.1)	20.8 (17.3–24.2)	15.6 \pm 0.90 (13.0–18.4)
Phenylalanine	29.1 \pm 0.43 (27.0–31.3)	29.6 (20.4–31.2)	35.1 \pm 0.88 (33.6–37.2)	33.2 (31.0–35.5)	29.6 \pm 1.81 (24.4–35.8)
Hydroxytyrosine	24.9 \pm 0.97 (18.3–29.6)	23.5 (20.0–24.9)	16.1 \pm 1.37 (12.3–20.4)	20.8 (20.3–21.4)	21.0 \pm 1.47 (16.7–27.2)
Lysine	20.4 \pm 0.99 (16.5–25.8)	22.0 (18.4–28.6)	34.1 \pm 1.70* (29.7–37.2)	28.1 (24.3–32.0)	28.4 \pm 2.36*** (21.7–36.0)
Histidine	14.5 \pm 0.61 (11.7–16.6)	13.2 (10.8–18.3)	16.8 \pm 1.17 (14.0–19.8)	15.7 (14.7–16.6)	15.3 \pm 1.00 (12.0–18.2)
Arginine	41.5 \pm 1.64 (34.5–51.7)	41.7 (38.1–45.8)	51.3 \pm 3.13 (42.2–60.6)	45.0 (40.0–50.1)	43.9 \pm 2.60 (31.9–49.3)
Half-cystine	26.1 \pm 1.63 (N=6) (22.0–32.9)	16.1 (N=1)	24.8 (22.2–26.2) (N=3)	18.8 (N=1)	8.1 (N=1)

For explanation, see footnotes to Table III. Residues per 1 000 amino acid residues (T-values) were calculated. *Without regard to tryptophan, which was not analysed.

sibly also of cholesterol seem to be slightly higher in GBM samples from diseased kidneys than from normal kidneys.

Amino acid composition. The amino acid composition is given in $\mu\text{M}/100\text{ mg}$ in Table III and in residues per 1 000 residues (T-values) in Table IV. In the CMPGN and IMGN groups, and to lesser extent the CPN group, the content of 3-hydroxyproline, 4-hydroxyproline and hydroxy lysine appeared to be reduced, and the content of lysine to be increased, but these differences were not obvious in the CGN group. Further dif-

ferences between the GBM samples prepared from the diseased kidneys and from normal kidneys were a moderately increased content of alanine in all the diseased kidney GBM preparations. In the CMPGN group a marked decrease was apparent for glycine and an increase was seen for aspartic acid, threonine, valine, tyrosine, phenylalanine and arginine. The mean ratio 4-hydroxyproline/proline was for the normal kidneys 1.31 ± 0.308 (mean \pm S.E.M., range 0.99–1.90) CGN 1.11 (1.10–1.12) CMPGN 0.63 ± 0.050 (0.55–0.84) IMGN 0.88 (0.81–0.93) and for CPN 1.03 ± 0

Table V Amino acid composition of lyophilized human glomeruli from four normal kidneys of sonication-resistant hyalinized, glomeruli from one kidney showing CPN and from one kidney showing CMPGN. Residues per 1000 amino acid residues (T-values mean and range)

	Normal kidneys	CPN	CMPGN
3-hydroxyproline	8.7 (7.2-11.9)	10.5	6.4
4-hydroxyproline	56.8 (46.1-76.1)	73.9	45.5
Aspartic acid	74.5 (64.0-82.3)	65.3	69.1
Threonine	40.4 (34.7-44.2)	31.4	35.6
Serine	51.5 (46.7-55.3)	44.9	45.4
Glutamic acid	100.0 (92.1-109.5)	87.5	90.5
Proline	78.3 (69.4-95.5)	91.1	78.0
Glycine	177.1 (160.4-214.5)	214.2	231.6
Alanine	72.4 (66.9-72.5)	74.4	67.3
Valine	31.1 (13.7-40.7)	37.2	33.6
Isoleucine	34.8 (29.8-39.5)	28.3	29.6
Leucine	72.2 (58.7-81.5)	56.8	60.2
Tyrosine	17.5 (14.1-19.6)	14.4	17.3
Phenylalanine	31.6 (26.0-40.0)	4.9	29.0
Hydroxylysine	11.1 (6.3-14.5)	15.1	14.7
Lysine	30.7 (16.6-39.9)	25.1	28.0
Histidine	16.5 (12.8-20.4)	12.6	13.0
Arginine	51.7 (49.1-56.4)	45.5	46.4

Methionine, cystine and tryptophan were not analysed. To facilitate comparisons with the amino acid analyses of GBM samples in Table IV it is assumed in the calculation of the T-values that the values for methionine and half-cystine were the same as normal GBM preparations, and no tryptophan was considered to be present.

(0.85-1.24). This ratio for CMPGN is significantly different from the ratio for normal GBM ($2p < 0.001$), while the ratio for CPN is not quite significantly different from the ratio for normal GBM ($0.05 < p < 0.10$). The ratio hydroxylysine/4-hydroxyproline for the different groups was: normal GBM 0.31 ± 0.015 (mean \pm S.E.M., range 0.1-0.37) CGN 0.29 (0.24-0.33) CMPGN 0.37 ± 0.040 (0.6-0.48) IMGN 0.37 (0.35-0.39) CPN 0.30 ± 0.030 (0.22-0.40). There is no significant difference between these ratios.

The amino acid composition of lyophilized whole human glomeruli, prepared from normal kidneys and of sonication-resistant glomeruli from one kidney in the CMPGN group and one in the CPN group is given in Table V for comparison.

The content of 3- and 4-hydroxyproline was lower in whole glomeruli than in normal GBM with a lower ratio of 3- to 4-hydroxyproline 0.15 compared to 0.22. The concentration of proline was higher and of glycine lower in the whole glo-

meruli. In particular the concentration of hydroxylysine was decreased and of lysine increased, giving a hydroxylysine/lysine ratio of 0.36, compared to 1.2. In normal GBM and a hydroxylysine/4-hydroxyproline ratio of 0.19 in glomeruli, compared to 0.30 in normal GBM.

The two preparations of hyalinized glomeruli analysed showed a 4-hydroxyproline concentration that was higher than in whole glomeruli but lower than in normal GBM. The glycine content was as high as, and the proline content even higher than, in normal GBM. The hyalinized glomeruli resembled glomeruli more than GBM with respect to their low ratio of hydroxylysine to lysine (0.60 and 0.53 respectively) and of 3- to 4-hydroxyproline (0.14 and 0.093 respectively) for the two preparations of hyalinized glomeruli.

Glucosyl-galactosyl-hydroxylysine. The GBM concentration of Glc-Gal-HyLys was in normal kidneys 72.3% (62.0-78.5) ($N=10$) of the total hydroxylysine in CGN 66.4% (61.9-74.8) ($N=3$) in CMPGN 68.1% (66.7-70.5) ($N=3$), in IMGN 69.5% (65.6-73.4) ($N=2$) and in CPN 65.0% ($N=1$). Thus in all the diseased GBM groups the percentage of hydroxylysine substituted with disaccharide was lower than in normal kidney GBM. When the whole group of diseased GBM was compared with normal kidney GBM the difference was found to be not significant.

Carbohydrate composition. Results of the analyses for hexoses, hexosamines, sialic acid and fucose are given in Table VI.

A strongly significant decrease in the concentrations of glucose and galactose was seen in the group CMPGN as compared to the normal group. A not significant increase ($0.05 < 2p < 0.10$) in glucosamine was also seen in this group. The remaining carbohydrate analyses did not show a clear difference between normal and abnormal GBM.

DISCUSSION

In all types of CGN increased thickening and irregularities of the basement membrane are common features. At the same time as these changes are seen increased glomerular permeability for macromolecules is present. In chronic pyelonephritis, on the other hand, changes in the GBM are seen only in glomeruli well on their way to be-

Table VI Carbohydrate composition of human GBM ($\mu\text{g}/\text{mg}$ mean \pm S.E.M. or range)

No. of samples analysed given in italics. All silicic acid analyses were carried out in duplicates. Analyses for hexosamines were carried out in duplicates for 10/17 samples

	Normal kidneys	CGN	CMPGN	IMGN	CPN
Glucose	23.0 \pm 0.26 J	25.4 (24.5-26.5) J	15.7 \pm 2.26 4*	NA	24.3 (21.4-27.2) 2
Galactose	29.6 \pm 0.28 J	23.7 (19.6-27.5) J	17.9 \pm 1.56 4***	NA	23.7 (22.7-4.8) 2
Mannose	6.1 \pm 0.39 J	5.5 (3.9-6.9) J	6.5 \pm 0.70 4	NA	6.8 (6.8-6.9) 2
Glicosamine ^a	16.2 \pm 0.43 (15.0-17.4) 6	14.8 (11.0-19.7) 2	19.0 \pm 1.21 (17.1-21.3) J	NA	23.2 (13.3-33.1) 2
Oelactosamine ^b	0.9 (0-2.0) 6	0.4 (0-0.8) 2	0.5 (0-1.6) J	NA	1.1 (0.5-1.6) 2
Silicic acid ^c	7.1 \pm 0.17 (7.1-8.5) J	7.0 (5.4-9.0) J	8.7 \pm 0.57 (6.9-10.2) J	7.7 J	7.7 (7.2-8.6) 4

Calculated as N-acetylglucosamine.

Calculated as N-acetylgalactosamine.

Calculated as N-acetylceramide acid.

NA = not analysed.

0.05 < 2p < 0.10, 0.02 < 2p < 0.05, 0.01 < 2p < 0.02,

2p < 0.01 compared with normal kidneys.

hyalized and even then these changes are usually not severe. In CPN only a very slight increase in the degree of proteinuria is observed.

Whether the increased glomerular permeability necessarily indicates a change in the permeability characteristics of the GBM is not clear. Increased excretion of GBM fragments in the urine, and excretion of abnormal GBM fragments, in experimental disease and in human glomerulonephritis, points to a change in the metabolism of GBM in these situations (5-9, 12).

In this study definite differences in the relative amounts of the amino acids were found between GBM from the diseased kidneys, particularly CMPGN and IMGN on the one hand and GBM from normal kidneys on the other. The amino acids known to occur only in collagens and basement membranes, 4-hydroxyproline and 4-hydroxylysine, were reduced in amount in GBM from the CMPGN and IMGN kidneys and to a lesser extent in GBM from the CPN kidneys, but not in GBM from CGN kidneys. 3-hydroxyproline, an amino acid not found in significant quantities in tissues other than basement membranes, may be taken as an indicator of a dilution of the normal GBM collagen-like constituent. The concentration of this amino acid was clearly reduced in the diseased GBM, with the possible exception of CGN GBM. Other amino acids, such as alanine, asine and phenylalanine, showed an increased concentration in GBM from CMPGN, IMGN and CPN kidneys.

In CGN an increase was seen for proline, glycine and alanine, a change that could be interpreted as evidence of contamination with collagen.

However the very small decrease seen in the concentration of hydroxylysine speaks against a significant content of collagen of typical amino acid composition.

The most striking differences in composition in both amino acid and carbohydrate composition were seen between the CMPGN group and normal GBM. In general the GBM from the CMPGN kidneys was more similar to whole glomeruli than to normal isolated GBM. As these isolated glomeruli contained relatively few cells, their chemical composition may to a large extent reflect a mixture of GBM and mesangial matrix. Material similar to that present in normal mesangium could very well be included in our GBM preparations from the CMPGN group. In the amino acid composition strong similarities are also apparent between GBM from CMPGN kidneys and the glycoproteins isolated by Kefauver (7) from dog or rat GBM after collagenase digestion. In general the GBM preparations in the CMPGN group show amino acid concentrations lying between the values for normal GBM and these isolated glycoproteins. As the glycoproteins contain almost no glucose, a GBM preparation containing an increased amount of glycoproteins and a correspondingly decreased amount of collagen should show a decreased ratio of glucose to galactose. This is not the case in the CMPGN GBM. It is likely that the differences seen between GBM preparations from normal kidneys and CMPGN kidneys represent the sum of more than one type of biochemical alteration.

The values observed for the two GBM samples in the IMGN group are similar to those obtained

for the CMPGN group but with generally smaller differences compared to normal GBM.

In the CPN group the values obtained for amino acid and carbohydrate concentrations are relatively similar to those for normal GBM but with lower concentrations of 3- and 4-hydroxyproline and hydroxylysine and higher concentrations of alanine and lysine. In these respects there are similarities between the CPN group and the samples of hyalinized glomeruli analysed. The pronounced decrease in the ratio of 3- to 4-hydroxyproline seen in the hyalinized glomeruli (0.098 compared to 0.22 for normal GBM) is more moderate in the CPN GBM (0.19). This, and the absence of an increase in the glycine content in the CPN group, speaks against contamination with collagen as an explanation of the change in composition.

Very few studies have been published on the chemical composition of GBM in experimental kidney diseases in animals (7). In a metabolic study of nephrosis induced in rats by the aminonucleoside of puromycin Blau and Michael (3) found a significant increase in the incorporation of labelled proline and conversion to labelled hydroxyproline in GBM with the onset of proteinuria. Their experiments also indicate that there is a pool of recently synthesized and rapidly catabolized glomerular hydroxyproline with an apparent half-life much shorter than the 12-13 days calculated from the later part of the activity slope. A previous report (8) of the isolation of a glycopeptide from rat GBM in aminonucleoside nephrosis, containing hydroxylysine, galactose and glucose in the ratio 1:1:1, could not be substantiated.

Beisswenger (1) reported preliminarily on the chemical composition of human GBM isolated from patients with several kinds of kidney diseases. The types of disease studied were not the same as in this paper. He found no difference in the amino acid composition of GBM from normal and diseased kidneys. Like Beisswenger and Spiro (2) Beisswenger (1) found in normal GBM a lysine/hydroxylysine ratio of over 1 and proline and hydroxyproline were present in almost equal molar quantities. In our study we also found an increased concentration of proline compared to hydroxyproline in GBM from diseased kidneys, particularly in CMPGN and IMGN.

Mahlen (10) has published a study on the chem-

ical composition of GBM isolated from six kidneys showing membranoproliferative or lobular glomerulonephritis. The amino acid composition of the diseased GBM in his study is quite different from our results. In fact his data show a striking similarity to our analysis of hyalinized glomeruli, particularly from the CMPGN kidney. Mahlen states that longer sonication times were often necessary for the abnormal kidneys. As apparently no attempt was made to remove the hyalinized glomeruli, they must have been included in the final preparation either disrupted or intact.

The main problem in the analysis of GBM isolated from diseased kidneys is the impossibility of obtaining preparations completely free of contaminating cellular and mesangial material. The intimate association between the plasma membranes of the endothelial and epithelial cells and the GBM will always raise the question to what extent the differences in chemical composition between GBM samples obtained from different groups of kidneys represent variations in contamination or a change in the composition of GBM proper. This is true in particular of the phospholipid and sialic acid values.

Contamination with mesangial material represents an even more difficult problem. There is no clear anatomical dividing line between the mesangium and the GBM. In CMPGN where mesangial processes appear to duplicate the basement membrane (11), no really reliable separation by physical means seems possible. A chemical study of the mesangial material would be of great interest. At present it is not known whether the mesangial extracellular material resembles GBM in its chemical composition or not.

Analyses of the different chemical building blocks in diseased GBM such as the two types of glycopeptides identified by Spiro (13), might offer the best possibility for an understanding of the nature of the chemical alteration of the GBM in different glomerular diseases. For such analysis the study of GBM from kidneys in various stages of disease would be of the greatest value. Until such studies have been undertaken the interpretation of the result of the present study must be done with great caution.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Diabetes Association, the National Institutes of Health

(AM 13756, AM 12375 HE 05662, HE 06134 II 1263C), the American Heart Association, the Minnesota Heart Association, and Förenade Liv Försäkringsbolag, Sweden.

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ALBUMINURIA IN ACUTE AND CHRONIC RENAL FAILURE

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Abstract Using radial immunodiffusion technique urinary albumin excretion has been studied in 28 patients with chronic pyelonephritis, 13 with polycystic kidney disease, 8 with gouty nephritis, 6 with acute renal failure, 31 with chronic glomerulonephritis, 4 with renal graft, and in 25 healthy volunteers. Expressed as the albumin/creatinine clearance ratio the excretion of albumin in the patients with non-glomerular renal disease, in those with renal graft showing no signs of rejection, and possibly also to some extent in those with chronic glomerulonephritis, varied severely with the glomerular filtration rate. Neither arterial hypertension nor urinary tract infection (past or present) seemed to have any effect on the excretion of albumin. In the investigation of urinary proteins the glomerular filtration rate should always be taken into account.

One of the classical signs of renal disease is proteinuria. Except for the situation in tubular disorders, myelomatosis and some rare conditions, the preponderant urinary protein is albumin. Traces of albumin normally occur in the urine (1) but the presence of notable amounts usually suggests glomerular disease with increased glomerular permeability to macromolecules.

But as is well known, the excretion of albumin may be increased also in non-glomerular renal disease, especially if the glomerular filtration rate (GFR) is markedly reduced. Neither the clinical significance nor the underlying mechanism of albuminuria in non-glomerular renal diseases is properly understood. This paper concerns albuminuria in non-glomerular renal disease and, for comparison, the excretion of albumin also in glomerular diseases. The investigation was undertaken in the hope that the findings might be of diagnostic interest and possibly also contribute to an understanding of the mechanisms of the excretion of albumin.

PATIENTS AND METHODS

The clinical material consisted of 65 patients with non-glomerular renal disease and 31 with glomerular disease.

This former group included no patients with disorders known occasionally to be associated with glomerular damage, such as systemic diseases, rheumatoid arthritis, scleroderma, vascular diseases other than arterial hypertension, or infections other than those of the urinary tract. Neither patients with suspect acute glomerulonephritis nor with the nephrotic syndrome in their history were accepted.

Chronic pyelonephritis Twenty-eight patients had chronic pyelonephritis. This condition was regarded as obstructive in 9 (2 with hyperplasia of the prostate and 7 with renal calculi) and as non-obstructive in 19 (11 of whom were abusers of analgesics containing phenacetin). All the patients in the obstructive group and 17 of those in the non-obstructive group had radiographically demonstrable scars in the renal parenchyma and papillary destruction of varying severity.

Two of the patients with non-obstructive chronic pyelonephritis had only negligible radiographic changes in the renal parenchyma. These patients were assigned to the pyelonephritis group because percutaneous renal biopsy performed to clear up the cause of unexplainable nitrogen retention, had shown severe interstitial nephritis. These two patients and five of the abusers of analgesics had no history of urinary tract infection, and urinary cultures performed at regular intervals for many years had never revealed bacteriuria.

Polycystic kidney disease (13 patients). This diagnosis was made on the basis of typical changes in I. pyelograms or renal angiograms, enlargement of the kidneys, which could be demonstrated by palpation of the abdominal wall, and the fact that all had one or more relatives with the disease.

Gouty nephritis (8 patients) Seven of the patients had history of recurrent attacks of typical gouty arthritis, not provoked by alcoholics, raised level of serum uric acid and delayed elimination of uric acid. The remaining patient had never had arthritis, but the serum level of uric acid had always exceeded 8 mg/100 ml despite only moderate reduction of the GFR, and percutaneous renal biopsy had shown severe interstitial nephritis.

Acute renal failure (6 patients). Three of the patients fulfilled Oken criteria for vasomotor nephropathy (10); the cause was crush syndrome in two and septic abortion in one. In one patient the cause of the renal failure was intoxication with multiple drugs and in one the cause was unknown, but renal biopsy had shown the

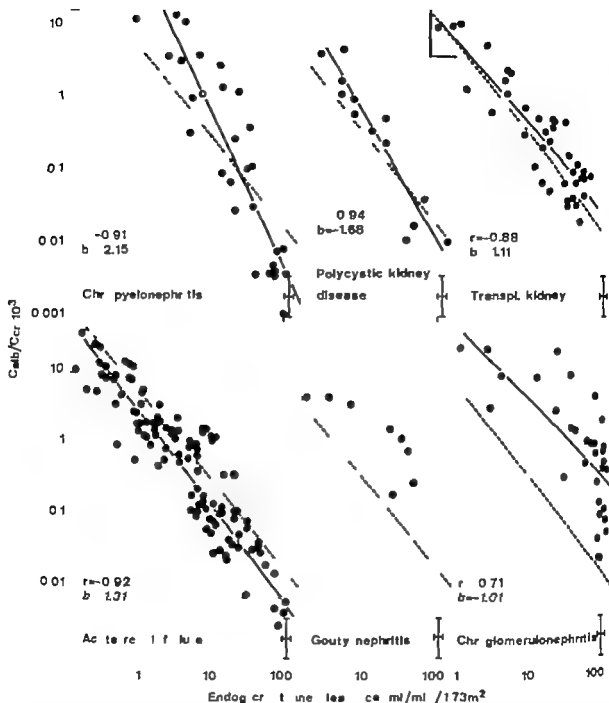


Fig. 1 Relation between C_{cr} and C_{ealb}/C_{cr} in 28 patients with chronic pyelonephritis, 13 with polycystic kidney disease, 8 with renal allograft (38 observations), 6 with acute renal failure, 8 with gouty nephritis and 31 with chronic glomerulonephritis.

— Regression line of each group calculated from the

log values; regression line calculated from the log values of all observations made on the non-glomerular renal disease groups. The significance of all regression lines was high ($p < 0.001$). The cross in the lower right-hand corner of each part of the figure—mean value of 25 controls \pm S.D.

typical histological features of acute tubular necrosis. The sixth patient had developed anuria after delivery followed by massive blood losses, and renal angiography on day 7 after the delivery had revealed bilateral cortical

necrosis with only small area of intact cortical tissue on one side. Within 6 months the GFR gradually increased to 18 ml/min while the GFR in the other five patients became practically normal within 2 or 3 months.

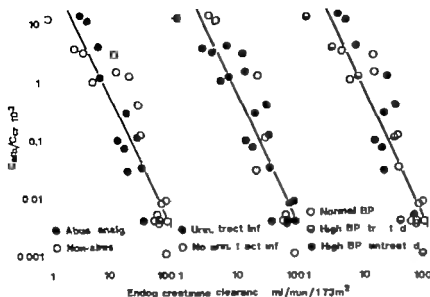


Fig. 2. Relation between C_{cr} and C_{125} , C_{cr} in 28 patients with chronic pyelonephritis.

All six patients had urine output of less than 100 ml/24 h for a week or more and all were dialysed at least three times.

Transplanted kidneys (8 patients). Urine was sampled repeatedly from the day of transplantation until the first signs of rejection. Only urine excreted by the graft was considered. Detailed information about these patients has been given elsewhere (14).

Chronic glomerulonephritis (31 patients). The diagnosis had been based on the clinical and laboratory findings and the microscopic appearance of percutaneous renal biopsy specimen. The cases were classified as proliferative or membranoproliferative glomerulonephritis (15 patients, including one with thromboses of the renal veins), membranous glomerulonephritis (6 patients), focal glomerulonephritis (2 patients), LED (4 patients), Henoch Schönlein's purpura (2 patients) or renal amyloidosis (2 patients).

Controls Twenty-five persons without symptoms or signs of renal disease served as controls. The criteria for selecting these individuals have been given elsewhere (6).

Dietary measures and calcium intake. The patients delivered 24-hour urine sample, just before or after the collection period, blood sample was obtained from each patient. Each sample was analysed for albumin and creatinine. The patients with renal graft and those with acute renal failure were repeatedly examined in this way. On the average five 24-hour samples were obtained from each member of the former group and 18 from the latter.

Albumin excretion was expressed as the ratio clearance of albumin clearance of creatinine (C_{ab}/C_{cr}) (4). Endogenous C_{cr} , ml/min/1.73 m² was calculated for the 24-hour sample used for albumin analysis and for at least one further 24-hour sample.

Analysed methods. Albumin was determined by radial immunodiffusion (9). Rabbit antiserum was supplied by C-B Laurell, Malmö, and commercial preparation of human albumin (Albumin 20% Kab) was used as

standard. Creatinine was measured by an aminolytic technique (3). The coefficient of variation of 20 duplicate determinations of albumin in the urine was 6.4%, of albumin in the serum 8.2%, of creatinine in the serum 2.9% and of creatinine in the urine 5.2%. The coefficient of variation of the equation C_{ab}/C_{cr} calculated from these duplicate analyses was 17.8%.

RESULTS

In all the groups a more or less close inverse relationship was found between GFR, measured as C_{cr} and C_{ab}/C_{cr} (Fig. 1). The logarithmic curve for this relationship was clearly linear in almost all of the non-glomerular groups, with small but sometimes significant differences between the slopes of the curves.

Chronic pyelonephritis. The sharpest slope between $\log C_{cr}$ and $\log C_{ab}/C_{cr}$ was seen in this group (-0.91 $b = -2.15$ $p < 0.001$). The patients were compared in four ways: abusers of analgesics versus non-abusers, patients with urinary tract infection (existing or earlier) versus those without, patients with untreated or treated arterial hypertension versus those with a normal arterial blood pressure, and patients with untreated hypertension versus those with treated hypertension and those with a normal blood pressure, but no significant differences between the slopes were found ($p > 0.1$ in all cases) (Fig. 2).

Polycystic kidney disease. The findings were principally the same as those in the former group ($r = -0.94$ $b = -1.68$, $p < 0.001$). The slope was

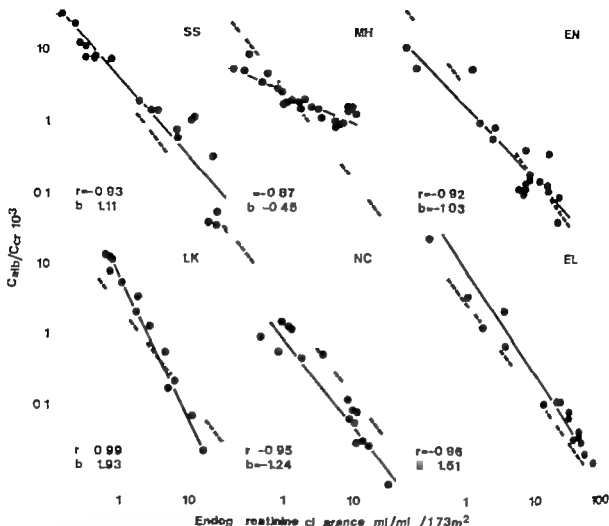


Fig. 3. Relation between C_{cr} and C_{alb}/C_{cr} in 6 patients with acute renal failure caused by crush syndrome (SS), septic abortion (NC), intoxication (LK), bilateral cortical necrosis (MH) and unknown reason (EN biopapillary necrosis).

— Regression line calculated from log values of each patient;
- - - regression line calculated from log values of all 6 patients.

not so sharp as in the pyelonephritis group, but the difference was not significant ($p > 0.1$).

Gouty nephritis. The number of patients investigated was not large enough to warrant statistical evaluation. However C_{alb}/C_{cr} seemed to be relatively high compared with that for the whole non-glomerular group.

Transplanted kidneys. Here again, an inverse, clearly linear relationship was found between $\log C_{cr}$ and $\log C_{alb}/C_{cr}$ ($r = -0.88$ $b = -1.11$ $p < 0.001$). The slope was significantly less sharp than in the patients with chronic pyelonephritis ($p < 0.001$) or polycystic kidney disease ($p < 0.05$).

Acute renal failure. The inverse relationship between $\log C_{cr}$ and $\log C_{alb}/C_{cr}$ was extremely close in each of the 6 patients, in 3 the regression coefficient was above 0.95 (values for the whole group $r = 0.92$, $b = -1.31$ $p < 0.001$) (Fig. 3). The slope of the curve for this group was less sharp than for the other groups, except for the patients with renal grafts, but differed significantly only from that for the pyelonephritis group.

Chronic glomerulonephritis. A negative correlation was found also in this group but with a wide range of variation ($r = -0.69$ $b = -0.82$ $p < 0.001$).

DISCUSSION

Platt et al. (12) found removal of renal tissue in the rat to cause significant and persistent increase of proteinuria, expressed as the rate of protein excretion per minute. They concluded that "since this came from a much smaller number of nephrons, the amount of protein being excreted per nephron must have been very greatly increased". They assumed that the proteinuria was due either to increased permeability of the glomeruli, enlarged after the reduction of renal mass, or to reduced tubular reabsorption of protein or to both.

The most striking finding in the present study was that the degree of albuminuria in the non-glomerular renal diseases and in the patients with renal grafts did not vary with the type of renal injury or the presence of complicating hypertension or urinary tract infection but was in close relation to the GFR. This is in accordance with the above mentioned findings in experimental uraemia and seems to lend further support to the view of Platt et al. (11) and Bricker (2) that in most diseases of the kidney the renal functional disturbances in chronic renal failure are similar and can be ascribed mainly to loss of nephrons.

Similar findings have been made in a study of the urinary excretion of the three low molecular weight proteins, α_2 -microglobulin, β_2 -microglobulin and lysozyme (4) and, less markedly in a study of two unidentified urinary proteins (5). Also these observations are in accordance with this theory though the elevation of the serum level of these proteins in proportion to the severity of uraemia may be a contributory factor.

The relation of the albuminuria with the severity of uraemia may be explained, at least partly by the assumption of decreased tubular reabsorption induced by uraemic toxins, or by degeneration of tubular cells. However impairment of tubular function may not be necessary for decreased reabsorption. If an increased load of albumin is presented to the tubules, fractional reabsorption of albumin per nephron decreases as a result. Such a mechanism might be expected, as a reduction of renal mass in experimental uraemia is followed by an increased GFR per nephron (12).

In acute renal failure and in patients with a renal graft the mechanism is more difficult to ex-

plain. If a uniform reduction of the GFR occurs in a large population of nephrons, it will presumably diminish the filtered load of albumin and lead to a relative increase in the reabsorption of filtered albumin and a corresponding decrease in the amount excreted, i.e. a decrease in C_{24h}/C_c . In fact, C_{24h}/C_c in these patients was relatively lower than in the other groups, but notably higher than in the controls. Tubular damage may be a contributory factor. Also humoral mechanisms may be involved, for proteinuria can be induced by epinephrine norepinephrine (7, 17) and renin (13), but its significance in acute or chronic renal failure is not known.

To exclude the possibility of complicating glomerulonephritis as the cause of the severe albuminuria in the "non-glomerular" renal diseases, histological studies of the glomerular structure would have been desirable. However percutaneous renal biopsy is not free from complications and was therefore not warranted in the cases with a firm clinical diagnosis. Though non-glomerular renal disease is occasionally combined with chronic glomerulonephritis or amyloidosis, it can hardly explain the present findings. An exception is gouty nephritis, which is often associated with glomerular changes typical of chronic glomerulonephritis (8, 16). Therefore in some of the patients with gouty nephritis and fairly severe albuminuria an increased glomerular permeability to albumin may have been a contributory cause of the albuminuria.

The correlation between C_c and C_{24h}/C_c was not so high in the glomerulonephritis group, probably because the critical factor in glomerulonephritis is an increased glomerular permeability which may vary independently of the GFR. But one might well imagine that the mechanism for the increase of C_{24h}/C_c in the non-glomerular group may also contribute to the albuminuria in glomerulonephritis, with reduction of the number of nephrons.

Whatever the cause of the findings, their practical implication is that the degree of albuminuria in non-glomerular renal disease and in patients with a renal graft showing no signs of rejection does not seem to furnish any information over and above that obtainable from determination of the GFR.

As a similar correlation has been found between the GFR and the excretion of all the

proteins hitherto studied (α_2 -microglobulin, β_2 -microglobulin, lysozyme (6), orosomucoid and IgG (15)) the GFR should always be taken into account in the investigation of urinary proteins.

ACKNOWLEDGEMENT

This study was supported by a grant from the Medical Faculty University of Lund.

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RENAL FUNCTION IN PATIENTS WITH UNILATERAL STAGHORN CALCULI

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Abstract The individual kidney function has been measured in seven patients with staghorn calculi in one kidney, the other kidney being normal. The calculous kidneys were all affected by pyelonephritis. The glomerular filtration rate was moderately reduced in the calculous kidneys. The functional pattern observed in these kidneys was that typical of pyelonephritis. This indicates that the presence of staghorn calculi per se either does not affect the renal function to any great extent or that it provokes functional patterns similar to that seen in pyelonephritis. The only moderate reduction of the function in the calculous kidneys indicates that nephrectomy should be the last therapeutic resort in these patients.

From clinical experience and reports on patients with affection of one or both kidneys (1-7) it is well known that staghorn calculi can reduce the glomerular filtration rate. A study of the characteristics of the functional derangement does not, however, seem to have been the object of earlier investigation.

Apart from the theoretical interest, such information might be of some practical importance as the presence of staghorn calculi often complicates the interpretation of renal functional studies in patients with pyelonephritis or when a nephron hypoperfusion syndrome is in question, such as in stenosis of the artery in an infected kidney.

The purpose of this study was to describe the functional pattern in patients with unilateral staghorn calculi and normal contralateral kidneys by the split function technique. The advantages of this experimental model, which provides an identical and normal interior milieu for each pair of kidneys, are self-evident. A similar study has not been published before.

MATERIAL

Seven patients were studied. The relevant data are given in Table I and Fig. 1.

The criteria for acceptance in the study are 1) the presence of staghorn calculi affecting at least 2/3 of all calyces of one kidney when examined by I. urography and 2) normal contralateral kidney. A kidney was considered as not affected when it was without clinical symptoms and signs of renal disease, and when the findings on uroscopical and bacteriological examination of the urine, on I. pyelography and—in most patients (Table I)—renal arteriography were normal. Patient 5 was an exception, having a small, superficial cortical cyst in the upper pole of the healthy kidney unobscured without any functional significance. The length of the non-affected kidneys was within 2 S.D. from the mean value of normal kidneys (2), i.e. gross compensatory hypertrophy was not present.

It will be seen that all kidneys with staghorn calculi were affected with pyelonephritis when the conventional criteria for this diagnosis are used (8). All biopsies are surgical. Urine aseptics from both kidneys and from the bladder were cultured for bacteria in all patients. The patients with an elevated blood pressure were all of the benign hypertensive type (6). All patients were without symptoms of circulatory insufficiency and the serum electrolytes were normal.

METHODS

The procedure for the selective renal function study and the analytical methods applied, as well as the method of measuring the diameter of the renal artery have been published earlier (3-4).

The figures in Table II are based upon the mean values of 4 clearance periods. In patient 5 there, as leakage to the bladder from the left ureter in the other patients no leakage from the ureters was observed.

The statistical significance of the difference between the normal and affected kidneys for the functions involved in this study was estimated by Student's *t*-test for paired samples.

RESULTS

The results are given in Tables I and II.

When the normal kidney with a cyst in the upper pole is excluded, the length of the affected

Table I. Relevant data on the patients studied

R = right, L = left, B = bladder BP = blood pressure first day in hospital

Pat. no.	Age (y.)	Sex	History (+/-)	Sediment (+/-)	Bacteri- uria (+/-)	Pyelo- gram (+/-)	Arterio- gram (+/-)	Diameter of renal artery (cm)	
								R	L
1	83	♀	+	B+	B+	L+			
2	45	♀	L+	B+		L+		6.3	5.6
3	32	♀	L+	B+	L+	L+	L+	7.6	5.4
4	27	♂	L+	B+	B+	L+			
5	69	♀	+	B+	B+	L+R+	L+	6.5	5.0
6	27	♀	R+	B+	R+	R+	R+	4.8	7.7
7	34	♀	L-	B+	L+	L+	L+	5.5	4.0

Cyst in right upper pole.

Table II. Function of the diseased (D) and control (C) kidney in the patients studied

Pat. no.	Diuresis (ml/min)		(Diuresis/Cl _{cr}) 100		Cl _{cr} (ml/min)		C _{PAH} (ml/min)		C _{crea} /C _{cr} (%)		C _{Na} /C _{cr} (%)		EF Na		EF-Na/ (C _{crea} /C _{cr})		FF	
	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C
1	2.1	2.3	4.5	3.6	23.3	32.2	79	98	5.2	4.0	3.9	3.1	4.2	3.9	8.8	1.0	0.29	0.13
2	1.7	3.7	5.7	3.7	29.5	90.5	154	499	1.7	1.2	3.7	2.5	0.7	0.5	0.4	0.4	0.19	0.22
3	1.6	1.0	6.0	1.0	26.5	93.5	123	478	4.2	1.8	1.9	[-0.7]	2.0	1.0	0.5	0.6	0.22	0.26
4	1.4	2.5	5.0	4.4	27.8	57.5	229	377	3.2	2.8	1.8	1.7	2.1	1.3	0.7	0.5	0.12	0.15
5	4.5	4.7	18.0	8.8	25.0	53.0	103	223									0.24	0.11
6	1.9	2.6	8.7	3.5	21.9	74.3	195	432									0.11	0.17
7	1.6	4.2	16.0	6.7	10.0	63.0	60	328									0.17	0.19
	2.1	3.0	9.2	4.5 ^{AB}	23.4	67.7 ^A	135	339 ^A	3.6	2.5	2.8	2.4 ^A	2.3	1.7	0.6	0.6	0.19	0.21

 $p < 0.02$

A: range of 1, 2 and 4.

kidneys was significantly less ($p < 0.02$) than that of the contralateral control kidneys; on average the difference was 1.0 cm. The diameter of the renal artery, the inulin clearance and the para-aminohippuric acid clearance were significantly less in the affected than in the non-affected kidneys ($p < 0.02$).

The diuresis, the free water clearance and the osmotic clearance were greatest in the affected kidney when related to the inulin clearance. The fraction of filtered sodium excreted (EF-Na) was also greatest in the affected kidney when related to the osmotic clearance/ml glomerular filtrate; this difference was no longer present. The difference in diuresis/ml inulin clearance was significant ($p < 0.02$); for the other parameters mentioned the observations were too few for statistical treatment of the results.

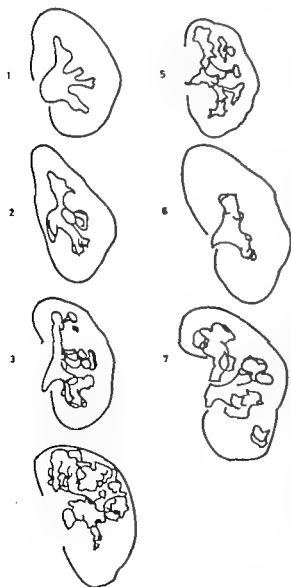
DISCUSSION

The functional pattern observed in the affected kidneys in this study is typical of that seen in pyelonephritis (5). As all kidneys with staghorn calculi were also affected by pyelonephritis, this indicates that the presence of a staghorn calculus per se either did not affect the renal function to any great extent or that it provoked a functional pattern similar to that seen in pyelonephritis.

The glomerular filtration rate was only moderately reduced even in the presence of staghorn calculi affecting all calyces. In fact six of the seven patients would have been able to live without symptoms of renal insufficiency even if the

Fig. 1 The affected kidneys with the staghorn calculi (1-7 = pat. nos.).

Kidney length (cm)		Cortical atrophy (0 to ++)	Caliculi size (0 to ++)	BP (mmHg)	Biopsy	Serum creatinine (mg)	Calices involved
R	L						
12	11	0	+	165/100		1.2	All
13	11	+	++	190/100	Fibrosis	1.8	All
14	12.5	++	++	190/110	Pyelonephritis	1.3	All
12.5	12.5	++	+	140/95	Pyelonephritis	1.4	2/3
16 ^a	12	+	0	225/120	Pyelonephritis	1.4	All
14	13	+	+	150/90		1.6	3/4
14.5	14	++	++	120/90	Pyelonephritis	1.1	1/4



contralateral healthy kidney for one reason or another had been destroyed. This is a strong argument against routine nephrectomy of kidneys with staghorn calculi and indicates that the treatment should be based upon removal of the stone and antibacterial therapy. After the stone has been removed the infection can be controlled in most patients, and even the renal function may improve (1-7).

It should be added that the study confirms the usefulness of measuring the diameter of the renal arteries when estimating the difference in function between two kidneys in one and the same individual (4).

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THE EFFECT OF INTRAVENOUS MORPHINE IN PATIENTS WITH MITRAL VALVULAR DISEASE AND CONGESTIVE HEART FAILURE

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Abstract. Thirteen patients with mitral valvular disease in cardiac functional groups III and IV were given 0.01 g morphine hydrochloride during diagnostic heart catheterization. The only significant hemodynamic effect was a fall in systolic blood pressure, which was most pronounced in cases with the highest pulmonary arterial pressures. A small, insignificant increase occurred in pulmonary arterial pressure, most marked in the cases with pulmonary hypertension. No significant effect on cardiac output or heart rate was observed after the injection of morphine. In all patients fall in arterial oxygen saturation was recorded, probably due mainly to simultaneous reduction of total and alveolar ventilation. The beneficial effect of morphine in congestive heart failure and pulmonary edema is probably due to variety of factors, venous pooling of blood, rise in pulmonary arterioles resistance protecting the alveoli against plasma transudation, sedative effect with reduced oxygen consumption and fall in alveolar ventilation. Finally fall in systolic arterial pressure reduces the tension time index and left ventricular work.

The main effect of morphine is a depression of the central nervous system which results in analgesia, sedation and respiratory depression. After 1 injection of morphine a peripheral release of histamine (11) and mobilization of catecholamines (14) from the adrenal medulla has been recorded. The chief hemodynamic effect seems to concern the peripheral circulation. Most important is probably a venous pooling of blood as demonstrated in dogs (5, 15) and by plethysmography in man (8). A marked tendency to orthostatic hypotension, which can be prevented by tourniquets applied to the lower extremities and relieved by elevation of the legs, has been documented (1). A transient fall in systemic blood pressure is often seen (3, 6), and this blood pressure reduction may sometimes be precipitous (13).

Morphine is an effective agent in the treatment

of acute pulmonary edema due to cardiac failure. The precise mechanism of this morphine effect is not exactly known. We have, therefore, wished to observe the hemodynamic effect in patients with congestive heart failure. In the present study an i.v. injection of morphine was given to patients with mitral valve disease in congestive failure during routine diagnostic cardiac catheterization. Prior to and following the administration of morphine hemodynamic and respiratory data were recorded.

MATERIAL AND METHODS

Thirteen patients are examined, all suffering from mitral valvular disease. Three also had aortic valvular disease. They were all in the cardiac functional groups III and IV according to the criteria of New York Heart Association, but the majority of the patients had improved somewhat during their stay in hospital prior to the catheterization study. Twelve patients had atrial fibrillation, only one had sinus rhythm. Nine were females, four males. Average age was 56 years (range 41-68).

Right heart catheterization was carried out with Cournand catheter and left heart catheterization with polyethylene catheter which was left in the aortic root. Pressures were measured with an Elema transducer. Cardiac output was calculated according to Fick principle. The volume of expired air was measured with Tissot spirometer and oxygen consumption was calculated from gas analysis by the micro-method of Scholander. Arterial pH and pCO_2 were measured by the Astrup technique. After completing the control measurements, 0.01 g morphine hydrochloride was injected intravenously into the catheter as bolus. The aortic pressure was continuously followed on the oscilloscope screen during the first 2 min. Later on, pressures were recorded every 2 min, and cardiac output 5 and 15 min after the injection. All patients are premedicated with allylpropylolol, 0.10 g, one hour before the procedure.

Table I. Hemodynamic effect (average) of i.v. injection of 0.01 g morphine in 13 patients with mitral valve disease

	Before injection	5 min after injection	15 min after injection
Cardiac index (l/min/m ² BSA)	2.2	2.2	ns
Stroke volume (ml)	51	46	ns
Heart rate /min	78	83	ns
Mean pulmonary arterial pressure (mmHg)	38	40	ns
Mean pulmonary wedge pressure (mmHg) ^a	19.6	20.2	ns
Pulmonary arteriolar resistance (dyn/sec cm ⁻⁵) ^b	263	289	ns
Mean aortic pressure (mmHg)	86	79	ns
Systolic aortic pressure (mmHg)	122	112 $p=0.05$	119
Systemic arterial resistance (dyn/sec cm ⁻⁵)	1937	1929	ns

^a Only 10 patients.

^b Average from 9 patients. The results from one patient, with pulmonary arteriolar resistance 1 408, 2 560 and 2 870 dyn/sec cm⁻⁵ respectively before, 5 and 15 min after injection, not included.

ns = not significant.

RESULTS

The hemodynamic effect of the morphine injection was usually moderate, and most marked during the first 5 min after the administration (Table I). Great individual variations were seen but, except for a fall in systemic arterial (aortic) pressure, no statistically significant changes were recorded in the patient group as a whole. During the first 5 min a slight reduction of stroke

volume was found probably secondary to a small rise in heart rate. Mean pulmonary arterial pressure increased in most patients with marked pulmonary hypertension but usually not in the cases with slight or no pulmonary hypertension (Fig. 1). Pulmonary wedge pressure usually did not change (Fig. 2). No significant change occurred in mean aortic pressure. In five patients, however a consistent fall in systolic aortic pressure, by 20% or more, was seen (Fig. 3). In three of these patients systolic aortic pressure fell after 5 min to 80 mmHg.

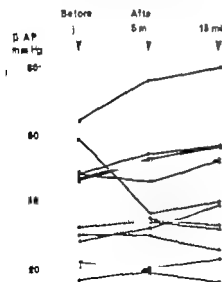


Fig. 1 Mean pulmonary artery pressure before, 5 and 15 min after i.v. injection of 0.01 g morphine hydrochloride.

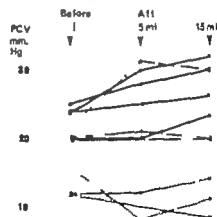


Fig. 2 Mean pulmonary wedge pressure before, 5 and 15 min after i.v. injection of 0.01 g morphine hydrochloride. — Patients in whom mean pulmonary artery pressure before the injection was <40 mmHg.

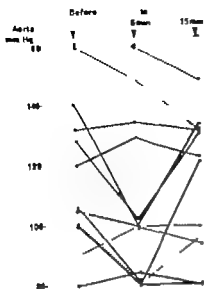


Fig. 3 Systolic aortic pressure before, 5 and 15 min after i injection of 0.01 g morphine hydrochloride. Symbols as in Fig. 2.

The patients were divided into two groups, one with marked pulmonary hypertension, with a mean pulmonary arterial pressure of 40 mmHg or more (6 cases), and one with normal pulmonary arterial pressure or an only slightly elevated level (7 cases) (Table II). The two groups reacted in a somewhat different way to the injection of morphine. Due to the small number of patients in each group statistical evaluation of the data

obtained has not been feasible. In the pulmonary hypertension group II c patients had a considerable further rise in pulmonary arterial pressure after morphine administration whereas usually no rise in pressure was seen in the normotensive group. The former group also seemed to have a slight tendency to a rise of the pulmonary wedge pressure and a greater tendency to a fall of the systolic systemic arterial pressure. The increase in heart rate and the fall in stroke volume was more pronounced in the patients with no or slight pulmonary hypertension.

The effect of the morphine injection on pulmonary ventilation was marked and highly significant (Table III). In this respect no difference was noted between patients with and without pulmonary hypertension (Table IV). Arterial oxygen saturation fell significantly and the reduction was most marked in patients with pulmonary hypertension (Fig. 4). During the first 5 min after the morphine injection a significant fall in oxygen consumption was seen most marked in the cases with pulmonary hypertension (Table IV). A moderate but significant reduction in base excess was also observed 15 min after the injection.

DISCUSSION

The most marked and only significant hemodynamic effect of an i. injection of 0.01 g morphine hydrochloride in the patient group as a whole was a fall in systolic systemic arterial pressure. This is in accordance with the initial

Table II. Hemodynamic effect of i.v. injection of morphine in six patients with severe pulmonary arterial hypertension, as compared with seven with no or moderate pulmonary arterial hypertension

	Mean pulm. art. press. (mmHg)	Before injection	5 min after injection	15 min after injection
Cardiac index (l/min/m ² BSA)	>40 <40	2.3 2.2	2.3 2.1	2.4 2.2
Stroke volume (ml)	>40 <40	46 35	45 47	46 53
Heart rate/min	>40 <40	83 74	85 82	82 81
Mean pulmonary arterial pressure (mmHg)	>40 <40	52 26	56 27	59 27
Mean pulmonary wedge pressure (mmHg)	>40 <40	24 17	25 18	31 17
Systolic aortic pressure (mmHg)	>40 <40	126 118	109 115	126 113
Pulmonary arteriole resistance (dyna/sec cm ⁻²)	>40 <40	427 181	423 233	509 194

Table III. Effect on the ventilation (average) of 1.5 injection of 0.01 g morphine in 13 patients with mitral valvular disease

	Before injection	5 min after injection	15 min after injection
Lung ventilation (l/min)	8.10	6.66	7.11
Alveolar ventilation (l/min)	4.14	3.14	3.60
pCO ₂ (mmHg)	36.3	39.7	39.1
pH	7.44	7.41	7.40
Arterial oxygen saturation (%)	91	86	86
Oxygen consumption (ml/min)	221	204	219
Standard bicarbonate	23.6	25.2 ns	24.2
Base excess	+1.5	+1.5	0*

$p < 0.01$ $p = 0.05$ ns = not significant.

hypotensive effect of morphine reported by others (3, 13). The systolic hypotension was most pronounced in patients with pulmonary hypertension. All these patients were on intensive diuretic therapy which may explain their tendency to fall in blood pressure. Severe hypotension, induced by morphine is probably more to be expected in patients with a normal or relatively low blood volume, as for instance in patients with acute myocardial infarction, as reported by Thomas et al. (13).

Pulmonary arterial pressure was usually additionally raised after morphine injection in patients with pulmonary hypertension, as has been previously reported by Fejfar et al. (2). In general, patients with no pulmonary hypertension show no

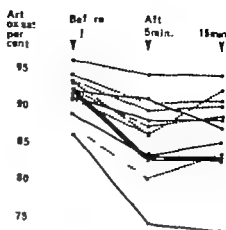


Fig. 4. Arterial oxygen saturation before, 5 and 15 min after 1.5 injection of 0.01 g morphine hydrochloride. Symbols as in Fig. 2.

rise of pulmonary arterial pressure. The different mode of reaction between patients with and without pulmonary hypertension is notable. This discrepancy cannot be related either to the degree of hypercapnia or to hypoxia. A study of patients with aortic stenosis has been reported in which morphine was found to evoke a moderate, but significant rise in pulmonary arterial pressure, whereas no such rise was observed in a non-cardiac patient (10). In normal dogs morphine did not give rise to any significant effect on the pulmonary arterial pressure (12).

Furthermore no significant effect on cardiac output was seen although great individual vari-

Table IV. Respiratory effect of 1.5 injection of morphine in six patients with severe pulmonary arterial hypertension, as compared with seven with no or moderate pulmonary arterial hypertension

	Mean pulm. art. press. (mmHg)	Before injection	5 min after injection	15 min after injection
Alveolar ventilation (l/min)	>40	4.40	3.13	3.23
	<40	3.95	3.15	3.84
pCO ₂ (mmHg)	>40	35.8	39.7	40.5
	<40	36.7	39.7	37.9
pH	>40	7.43	7.41	7.40
	<40	7.44	7.42	7.40
Arterial oxygen saturation (%)	>40	90	84	83
	<40	92	88	89
Oxygen consumption (ml/min)	>40	19	195	206
	<40	221	211	231
Base excess	>40	+2.7	+1.4	+1.4
	<40	-1.5	+1.6	-1.1

bloods were observed. This seems largely to be in accordance with data reported by others. Fejfar et al. in an examination of patients with mitral valvular disease have pointed out that cardiac output usually increases in patients with mitral valvular disease with low cardiac output prior to the morphine injection (2). The present study does not confirm this observation. An insignificant increase has been recorded in patients with acute myocardial infarction (4-13). Lowenstein et al. observed a significant rise in patients with aortic stenosis, but no change in normal subjects (10). In dogs an increase in cardiac output has been found, related to the release of catecholamines from the adrenals (14). Other investigators, however have observed a reduction in cardiac output in dog experiments (12).

Heart rate is usually reported to be unchanged after administration of morphine (10-13). In non-cardiac patients during operations a fall has been observed (3). The insignificant rise seen in the present investigation may probably be due to arterial hypoxia induced by morphine. An insignificant increase of pulse rate after morphine has also been reported in patients with mitral valvular disease (2).

In the present study injection of morphine gave rise to a fall in arterial oxygen saturation in all cases. In some patients the arterial hypoxemia was of unquestionable clinical importance. One half of the patients, however had a slight arterial hypoxemia also before the morphine injection. In a study on patients with acute myocardial infarction morphine induced arterial hypoxemia in patients with pulmonary congestion but not in the other cases (7). The hypoxemia is probably related mainly to the reduced ventilation. A slightly increased arterio-venous oxygen gradient has, however also been reported after administration of morphine (9). This factor may have contributed to the relatively marked hypoxemia seen in some of the patients in the present investigation. The clinical conclusion which may be drawn is that morphine should never be given to patients with congestive cardiac failure unless oxygen is simultaneously administered.

All the patients received diuretic therapy and had a slight base excess before the investigation. The base excess was abolished 15 min after the morphine injection, probably due to a slight metabolic acidosis related to the hypoxemia.

The beneficial effect of morphine in pulmonary edema is probably due to multiple factors, of which the following seem to be of greatest importance.

- 1 Venous pooling of blood, as has been demonstrated in dog (5) as well as in man (1-3).
- 2 Rise in pulmonary arteriolar resistance which will protect the alveoli against transudation.
- 3 Sedative effect with reduced oxygen consumption as observed in the present investigation.
- 4 Reduced ventilation. The forced ventilation in acute pulmonary edema probably leads to great fluctuations in the intrathoracic pressure, which promote transudation into the alveoli.
- 5 Fall in systolic aortic pressure, and thus a fall in tension-time index and left ventricular work.

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ASSESSMENT OF PROGNOSIS IN ACUTE MYOCARDIAL INFARCTION BY COMPUTER ANALYSIS OF CARDIO-RESPIRATORY VARIABLES

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Abstract Pressures in the radial and pulmonary arteries have been measured continuously and analyzed off-line on computer in 19 patients with acute myocardial infarction. Eight patients died from cardiogenic shock. Cardiac output, blood gases and acid-base balance were determined intermittently. The prognostic values of 4 variables were compared. Combinations of variables were studied by linear discriminant function analysis. The most reliable variables for prognostic purposes were stroke power index (SPI), left ventricular work index (LVWI) and the maximal derivative of the radial artery pulse wave ($(dP/dt)_{max}$). Pulse pressure, stroke work index and systolic pressure in the radial artery showed less discriminating power. Poor discriminating ability was observed for, inter alia, systolic ejection time, peripheral vascular resistance index, central aortic pressure, tension time index and arterial oxygen tension. Mean SPI was 1.58 W/m^2 in survivors and 0.87 W/m^2 in patients who died. For $(dP/dt)_{max}$ the corresponding figures were 1.23 mmHg/msec and 0.67 mmHg/msec , respectively. Separation between the two groups was not significantly improved with combinations of two or three variables as compared to SPI, LVWI or $(dP/dt)_{max}$ alone.

In spite of modern coronary care the mortality rate is still high in patients with acute myocardial infarction (AMI). It has long been recognized that the mortality rate is influenced by a number of factors such as a history of previous infarction, hypertension, high age, arrhythmia, conduction disturbance, diabetes and angina pectoris (4, 15). Other investigators have studied hemodynamic variables in AMI and correlated these with the outcome (14, 16). A method for the prediction of shock complicating AMI has recently been advised (8). Several of the reported studies have been performed with the use of computer technique.

In AMI the fundamental circulatory disturbance is caused by an impaired myocardial function and the degree of impairment is correlated to the course of the disease (11).

The aim of the present investigation was to analyse the prognostic value of different cardio-respiratory variables using computer technique. Peripheral arterial pressure was used for approximate evaluation of left heart function. Some of the variables used in this study have not been reported earlier for estimation of left ventricular (LV) function in AMI.

MATERIAL

Nineteen patients with typical history of AMI and with diagnostic ECG changes have been studied (Table I). For the statistical evaluation the patients have been divided into two groups: survivors (nos. 1-11) and non-survivors (nos. 12-19). In the surviving patients measurements were made 1-6 days following diagnosis of AMI. The non-surviving patients were studied 1-6 days after AMI and 1 hour to 13 days before death. Among the survivors 3 known base deaths have occurred. Patients 1 and 10 died from reinfarction 38 and 59 days, respectively after the acute episode. Patient 1 succumbed after more than 3 months in severe heart failure. Mean age for non-survivors was 64 as compared to 59 years for survivors. This difference was not significant. Among non-survivors history of hypertension and angina pectoris was more common. Five patients died in the coronary care unit (CCU) 2-4 days after admission. Four of the latter suffered cardiogenic shock and pulmonary edema before death. The fifth died from cardiac rupture 8 days after admission. Patient 15 died unexpectedly in the ward and had had clinical signs of myocardial insufficiency. Patients 16 and 19 died from pulmonary edema and irreversible extracardial fibrillation, respectively. Patient 18 was resuscitated on admission, but otherwise no potentially fatal arrhythmia occurred in the CCU before death. Autopsy showed extensive fresh myocardial damage in non-surviving patients. Patient 14 also had signs of previous infarction at autopsy.

METHODS

All patients were examined in the supine position. The mid-thoracic level was used as reference level for pres-

Table 1. Anthropometric and some clinical data of the 19 patients studied

BSA = body surface area, A = anterior wall, P = posterior, L = lateral, S = septal

Pat. no.	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m ²)	Localization of infarction in ECG	Max. SGOT	History of previous disease
1		67	179	60	1.76	P+L	30	Diabetes + myocardial infarction
2	♂	54	177	58	1.70	A+P	320	
3	♂	66	170	66	1.74	A	276	
4	♂	61	165	64	1.70	A	285	
5	♂	65	186	92	2.16	A	280	
6	♂	48	196	87	2.20	A	410	
7	♂	44	184	87	2.10	A	20	
8	♂	56	183	72	1.92	A+S	185	
9	♂	65	175	61	1.74	P+L	250	Suspected myocardial infarction
10		67	160	52	1.52	P	600	
11	♂	60	179	75	1.94	A+L	320	
12		51	164	53	1.56	A+S	660	
13	♂	67	164	68	1.74	P+L	29	Myocardial infarction + hypertension
14		65	158	78	1.79	A+S+P	320	Hypertension
15	♂	76	169	66	1.75	P+L	200	Angina pectoris
16	♂	70	176	72	1.87	A+L	184	Angina pectoris
17		76	174	64	1.77	A	96	
18	♂	56	177	56	1.68	A+L	334	Angina pectoris
19	♂	5	180	52	1.69	S+A+L	320	Angina pectoris

sure recordings. ECG, radial artery pressure (RAP), superior vena cava pressure (CVP) and pulmonary artery pressure (PAP) are all recorded simultaneously and stored on magnetic tape. Cardiac output (CO), arteriovenous oxygen difference (AVD) and arterial acid-base values were measured intermittently. For CO determination bolus of the indocyanine green as injected into the pulmonary artery. D₂-dilution curves were registered on Beckman cardiodensitometer and CO as determined by conventional methods.

Oxygen saturation was determined spectrophotometrically (CO-oximeter IL 187). Blood gas tensions and acid-base values are determined in arterial blood using Radiometer B3153 apparatus. Oxygen (O₂) uptake was calculated as the product of AVD and CO. Arterial temperature +0.3°C was taken as body temperature (BT). Heart rate (HR) was obtained from the R-R intervals of the ECG. ECG and pressure recordings are analysed in computer (IBM 1800) and average values over 20-sec periods are determined. Good agreement between computer and manually obtained data has been found (2). The RAP and PAP recording systems showed flat amplitude responses up to 8-10 Hz. Including some complex variables, total of 4 variables were studied.

The maximal derivative of the RAP curve ($(dp/dt)_{max}$) was computed. Systolic ejection time (SET) was calculated as the time distance between the foot point and measure of the arterial pressure curve.

Cardiac index (CI) as calculated from the expression $CO/(BSA)^{0.75}$ and stroke index (SI) from the expression $CI \times HR$.

Mean systolic ejection pressure (MSEP) as computed as mean pressure during SET.

Total peripheral vascular resistance index (PVRI) was calculated from the formula $(mean\ RAP - CVP)/CI$.

Tension time index (TTI) as calculated from the expression $MSEP \times HR \times SET$.

Left ventricular work index (LVWI) was obtained from the formula $(MSEP - diastolic\ PAP) \times CI$, stroke work index (SWI) from the formula $LVWI \times HR^{-1}$ and stroke power index (SPI), i.e. estimated left ventricular net pressure time during systole, from the formula $SWI \times SET^{-1}$.

Statistical analysis

For each variable the significance of difference between the means of the two groups was evaluated by two Student's *t*-test. The midpoint between the estimated means as used as reference value for classification. For further separation of the two populations, last discriminant function analysis as performed using about combinations of two and three variables. As measure of the separation achieved between survivors and non-survivors Mahalanobis D^2 (D^2) as used. Assuming normally distributed variables and identical prior probabilities, the probability of correct classification was estimated. The mathematical basis of discriminant function analysis has been described in detail by others (1).

RESULTS

Intravascular pressures

Mean values and standard deviation for the two groups of patients are presented in Table II. It appears that only pulse pressure and systolic RAP

Table II Mean values of 24 cardio-respiratory variables and probability of correct prediction of outcome in 18 patients with AMI

SRAP—systolic radial artery pressure, SPAP—systolic pulmonary artery pressure, DRAP—diastolic radial artery pressure, DPAP—diastolic pulmonary artery pressure, MRAP—mean radial artery pressure, MPAP—mean pulmonary artery pressure, PP—pulse pressure

Variable	Survivors (S) Mean \pm S.D. (n=11)	Non-survivors (N) Mean \pm S.D. (n=7)	Significance of difference between S and N ^a	D ^b	Probability of correct prediction of outcome
SRAP (mmHg)	131 \pm 16	111 \pm 19	0.05	1.27	0.71
DRAP (mmHg)	70 \pm 9	65 \pm 13	n.s.	0.19	0.39
MRAP (mmHg)	91 \pm 10	81 \pm 14	n.s.	0.61	0.65
PP (mmHg)	61 \pm 13	46 \pm 11	0.05	1.60	0.74
SPAP (mmHg)	32 \pm 7	38 \pm 11	n.s.	0.35	0.62
DPAP (mmHg)	14 \pm 6	20 \pm 9	n.s.	0.70	0.66
MPAP (mmHg)	22 \pm 6	28 \pm 9	n.s.	0.69	0.66
CVP (mmHg)	6 \pm 2	8 \pm 3	n.s.	0.56	0.65
HR (min ⁻¹)	94 \pm 16	103 \pm 19	n.s.	0.32	0.61
CI (l min ⁻¹ m ⁻²)	2.4 \pm 0.7	1.9 \pm 0.4	n.s.	0.66	0.66
SI (ml m ⁻²)	26 \pm 8	19 \pm 5	n.s.	0.96	0.69
AVD (ml l ⁻¹)	65 \pm 12	74 \pm 14	n.s.	0.49	0.64
O ₂ uptake (ml min ⁻¹)	284 \pm 80	249 \pm 62	n.s.	0.28	0.61
BT (°C)	37.4 \pm 0.7	37.4 \pm 0.8	n.s.	0.001	0.51
PaCO ₂ (mmHg)	33 \pm 8	34 \pm 7	n.s.	0.01	0.52
PaO ₂ (mmHg)	71 \pm 20 ^b	60 \pm 16 ^c	n.s.	0.38	0.61
BE (mEq l ⁻¹)	0.4 \pm 4.1	2.4 \pm 5.7	n.s.	0.17	0.58
SET (dP/dt) _{max} (mmHg sec ⁻¹)	1.25 \pm 0.26	0.87 \pm 0.27	0.01	1.92	0.76
dP/dt (mmHg sec ⁻¹)	0.23 \pm 0.03	0.22 \pm 0.03	n.s.	0.20	0.59
TTI (mmHg sec min ⁻¹)	2.343 \pm 301	2.135 \pm 351	n.s.	0.24	0.60
PVRI (dyn sec cm ⁻⁵ m ²)	3.179 \pm 1.579	3.104 \pm 3.46	n.s.	0.004	0.51
LVWI (W m ⁻²)	0.49 \pm 0.14	0.33 \pm 0.09	0.01	1.99	0.76
SWI (J m ⁻²)	0.33 \pm 0.14	0.19 \pm 0.05	0.05	1.53	0.73
RPI (W m ⁻²)	1.38 \pm 0.39	0.87 \pm 0.27	0.01	2.14	0.77

n.s. = not significant. ^a = 10. ^b = 7

separated the two groups ($p < 0.05$). For the pressures of the lesser circulation no significant differences were found. Diastolic PAP was elevated above normal in 6 of the 11 surviving patients and in 7 of the 8 non-surviving patients.

Blood flow measurements

HR and AVD were increased as compared to a material of normal men of corresponding age (3). Both CI and SI were low in comparison with this material. Body temperature was normal for both groups, but the calculated relative O₂ uptake was slightly increased, with a small difference between the two groups.

Blood-gas and acid-base measurements

Two patients received artificial ventilation with Engstrom respirators. Spontaneous respiratory rate was not monitored. Judged from PaCO₂ values there were no differences in alveolar ven-

tilation between survivors and non-survivors. Neither did PaO₂ or base excess values separate the two groups.

Computed variables

SET, TTI and PVRI were of the same range in the two groups. PVRI was slightly increased in a majority of patients. SPI, (dP/dt)_{max} and LVWI were considerably higher in surviving patients, and this difference was significant.

Discriminating ability for single and complex variables

Table II gives D² and the estimated probabilities for correct prediction of outcome for the variables studied. The intervals for predicted survival are given for each variable. The most reliable variables for prediction of outcome ranked by falling D² values were SPI, (dP/dt)_{max}, systolic RAP, mean PAP and diastolic PAP. Very low D² i.e. little or no discriminating ability was found for

PaO₂, TTI, PVRI, BT, diastolic RAP, SET and O₂ uptake.

Mean SPI was 1.38 W/m² in survivors and 0.87 W/m² in patients who died. For $(dP/dt)_{max}$ the corresponding figures were 1.23 mmHg/msec and 0.87 mmHg/msec, respectively. Separation between the two groups was not significantly improved with combinations of two or three variables as compared to SPI, LVWI or $(dP/dt)_{max}$ alone.

DISCUSSION

A reliable variable or index for estimation and monitoring of cardiac function should be sensitive to changes in cardiac performance. Some variables may be little influenced by nervous, hormonal or therapeutic effects. Other variables, however, are changed in order to compensate for the decreased cardiac function either actively e.g. HR and arteriolar tone or passively e.g. diastolic PAP and AVD. For most variables presented in Table II a combination of active and passive influences can be anticipated.

Among conventional monitoring data, such as systemic arterial and venous blood pressure and HR, only systolic arterial blood pressure was useful for prediction of prognosis in the present material. Automatic data processing of cardiovascular variables makes it possible to obtain other complex variables which on theoretical grounds may give better information of cardiac function. This investigation shows that some of these complex variables are more reliable for prediction of outcome in AMI than conventional monitoring data. The relatively small number of patients in the present study will result in some overestimation of the predictive ability when applied in a prospective study.

For evaluation of LV function it is desirable to measure pressures in the left heart or aorta. Such measurements may be difficult to carry out and also imply some risk for the patient.

In our calculation of external left ventricular work certain assumptions are made. Kinetic energy is considered negligible. Mean radial artery pressure during systole is considered to reflect the intraventricular pressure with acceptable accuracy. However, it is well known that peripheral systolic arterial pressure may exceed the maximal intraventricular pressure and thus introduces a certain error into the calculations. Factors such as in-

creases in vascular tone and HR may enhance the difference between centrally and peripherally measured pressures (9). Pulmonary diastolic pressure is a satisfactory indirect measure of mean left atrial pressure when pulmonary vascular resistance is normal (6). In peripheral recordings the inflexure of the arterial pressure curve occurs at a lower pressure and later than in central recording. This results in an increase of SET and probably some underestimation of SPI. Arterial hypotension and a low C.O. may also influence the peripherally recorded pressure curve. With a low C.O., as may occur in patients with myocardial infarction, the dye-dilution method may give a lower value for the C.O. as compared to the Fick method. Since the overall effects of these influences on SPI are not completely known, SPI should be regarded as partly empirical. The same applies to $(dP/dt)_{max}$. Despite this, SPI and radial arterial $(dP/dt)_{max}$ were superior to other variables for prediction of prognosis, a finding that has not been reported earlier in patients with AMI.

Scheidt et al. (14) have recorded LV pressures in AMI and reported values of LV work (LVW). In their material of 38 patients LVW for surviving patients was 0.95 W and for non-surviving 0.34. In the present study these values were 0.94 W and 0.58 W respectively. In this study SPI gave a better prediction of the outcome than LVWI.

Maximal dP/dt which separated the two groups almost as well as SPI may possibly be obtained by non-invasive methods. Kendi et al. (7) have investigated the hemodynamic effects of experimental myocardial infarction in dogs. They found that mean blood flow acceleration in the aorta was a very sensitive index of LV function. This supports our findings concerning $(dP/dt)_{max}$ since this factor apart from being influenced by peripheral resistance and HR, depends on SV and flow acceleration in the aorta. The area of a decreased myocardium in AMI will act as a damping element and thus reduce flow acceleration in the aorta.

In a study of 18 patients with AMI and cardiogenic shock Schubert et al. (16) have reported a mortality rate of 70%. Prognostic indices were calculated by discriminant function analysis. Left heart pressures were not measured or approximated, and consequently LVW could not be estimated. Schubert et al. found that C.O. and SV predicted the outcome better than systolic arterial

pressure. A combination of SI and diastolic artery pressure gave a significantly higher prognostic index than each variable separately. In our material, however, no combination of variables was significantly better than SPI alone. This can probably be explained by the fact that SPI in itself contains several parameters related to LV function.

A great number of variables had little or no prognostic significance, e.g. PVRI and CVP. These variables partially reflected the tone of resistance and capacitance vessels. The prognostic value is probably lessened by intervention of the cardiovascular regulating systems of the body. The uselessness of CVP measurement for evaluation of LV function in AMI has also been reported by other authors. Therapeutic measures such as administration of oxygen sodium bicarbonate or diuretics may lessen differences between the two groups for some variables.

TTI has been shown to correlate with oxygen consumption of the normal heart. TTI calculated by use of peripheral arterial pressure lacked prognostic value in this study.

SET estimated from the peripheral arterial pulse wave showed little prognostic value. In myocardial insufficiency SET is shortened in central or intraventricular measurements. Samson (13) investigated changes in systolic time intervals, among which SET in AMI. He found a decrease of SET in patients with AMI and suggested that this variable could be used as an index of myocardial function in AMI and possibly also give information concerning stroke volume. Jain and Lindahl (5) ascribed the shortening of SET to an increase in the amount of circulating catecholamines. Weisker et al. (17) found a good correlation between SET and CI. In our material the correlation coefficient between SI and SET was 0.71. A review of the use of systolic intervals in patients with AMI has recently been published (10).

Rutherford et al. (12) found in 25 patients with AMI that mean PAP rose before other clinical and laboratory investigations showed signs of myocardial failure. They considered PAP measure a reliable index of impending LV insufficiency and an excellent guide to therapy. Regarding the outcome we found a markedly better discrimination for SPI and $(dP/dt)_{max}$ than using mean PAP.

Changes in various cardio-respiratory indices during the course of AMI are important for the outcome and early detection of impaired cardiac function. Regarding SPI and $(dP/dt)_{max}$, lower values were found in patients studied within 4 hours before death. As a guide to treatment it may be important to follow changes in SPI and/or $(dP/dt)_{max}$ since a decrease in these variables may be an early sign of impending cardiogenic shock.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Board for Technical Development (89-1204 U 881 b), the Swedish Medical Research Council (B72-40X-679-07) and the Swedish Hospital Planning and Rationalization Institute (314).

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VARIATION OF QRS AMPLITUDE IN EXERCISE ECG AS AN INDEX PREDICTING RESULT OF PHYSICAL TRAINING IN PATIENTS WITH CORONARY HEART DISEASE

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Abstract. Several QRS amplitude changes taking place during exercise testing were used to derive an R wave variation index. This index was determined from the exercise ECGs of 28 patients who had sustained myocardial infarction before starting supervised physical training programme of 3 months. R wave variation index correlated with the ability to later increase of physical working capacity ($r=0.66$, $p<0.001$); the smaller the dynamic changes of the QRS amplitude during the initial exercise testing, the worse was the training result. The initial physical fitness and the maximum heart rate achieved during exercise testing had scarcely any predictive power in regard to the success of the subsequent training. The R wave variation index may be related to the hemodynamic capacity of the left ventricle and provides useful adjunct in applying training programmes.

Physical training of patients with ischemic heart disease has recently won great, and perhaps over emphasized, popularity. However despite regular training these patients do not always increase their physical working capacity (PWC). This inadequate response is understandable if the maximal training heart rate is greatly limited by causes such as severe angina pectoris, heart failure or claudication. Some patients, however despite reaching high training heart rates, still show little or no change in PWC and cardiovascular adjustments (15). These findings suggest that there are other factors related to the ability of gaining conditioning response.

Changes in the amplitude of the QRS complex in the exercise ECG recorded before starting of the training programme appear to be related to the success of physical training in patients who have sustained a myocardial infarction. The small variation in the amplitude of the QRS complex

during exercise testing, the worse is the training result. The predictive value of this finding for the training response is reported.

SUBJECTS

The group of patients with coronary heart disease (CHD) consisted of 28 men who had experienced acute myocardial infarction 6-8 weeks earlier. These patients belonged to series of 77 consecutive infarction patients who were included in the training programme (10). Their mean age was 52.6 years (range 39-64). Only these 28 patients faithfully attended supervised training sessions during 3 months.

The QRS variations during exercise test were also studied in group of 10 healthy male volunteers with a mean age of 41.1 years (range 26-54). These men were free of symptoms of ischemic heart disease and had normal resting and exercise ECGs.

METHODS

The exercise test was performed with an electrically braked ergometer (Eastronema Lode N.V. Groningen). The load was increased stepwise at 4-min intervals up to the maximum work level of the patient (10). ECGs are recorded during exercise with direct writing ink-jet 6-channel recorder (Elema-Schöninger). The recorder has linear sensitivity up to 500 cps. Before and after the exercise test ECG leads VI-V6 were recorded with the subject in the supine position, while leads CH1-CH6 were recorded when the subject was sitting on the bicycle before and during exercise.

For the present purpose the height of the R wave was measured from lead V3 recorded at rest in the supine position before and 1 min following the exercise. The difference between these measurements was expressed as percentage change. Similarly percentage change was measured from lead CH5 between heights of R wave recorded with the subject sitting on the bicycle before exercise and

Mean heart rate immediately after bicycle ergometer exercise, beats/min

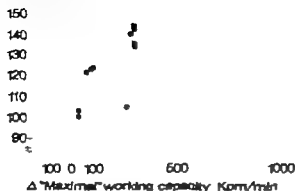


Fig. 1 Mean training heart rates of 28 infarction patients plotted against changes developed in PWC during the 3-month training programme.

highest R wave amplitude developing during exercise. In order to avoid the influence of respiration on the QRS amplitude, a mean value of R wave heights during inspiration and expiration was used in the calculations. An

R wave variation index¹⁰ was derived from the percentage figures by adding these signed values. A plus sign was used for the following normal responses: decreased R wave in V5 recorded in supine position and for increasing R wave in CH5 recorded on the bicycle. Changes opposite to these were signed negative. For example: decrease of R wave height in lead V5 by 10% from supine rest before exercise to supine rest after exercise, combined with a 5% increase in CH5 from sitting on bicycle to exercise slope, gives an R wave variation index of +15.

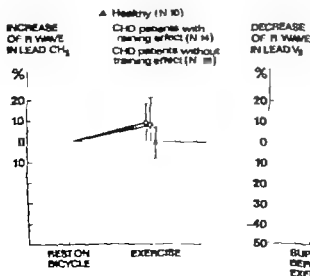


Fig. 2. Percentages QRS amplitude changes during exercise rest in healthy subjects (greatest changes) in patients with CHD showing good response to exercise, and in CHD patients without training effect (negligible changes).

PHYSICAL REHABILITATION PROGRAMME

The 28 patients with myocardial infarction attended regularly twice a week supervised physical training sessions, lasting 45 min each. After warming-up period the training programme consisted of heavy dynamic work interrupted by intervals of easier calisthenics, relaxing and breathing exercise. The intensity of strenuous exercise performed for 20 min was regulated so as to reach and keep the heart rate at level of about 10 beats/min lower than the highest rate recorded during the first maximum work test performed before the start of the training programme. This second work test was performed at the end of the training period 3 months after the initial one, and the change in PWC was calculated. The mean training heart rates were calculated from immediate postexercise heart rates recorded in connection with the ten last training sessions.

RESULTS

The mean training heart rate of the infarction patients did not correlate remarkably with the change in PWC occurring during the 3-month training period (Fig. 1). Notably the increment of PWC remained below 200 kpm/min in half of the patients despite the fact that many of them had a quite high training heart rate.

In the group showing little or no change in PWC, the variation of the R wave in either direction was negligible (Fig. 2). On the contrary in the group with a clear-cut increase of the working capacity the increase of the R wave in lead CH5 at the start of exercise was significant ($p < 0.05$) and the postexercise decrease of the R wave amplitude in lead V5 was even more marked ($p < 0.01$).

These R wave responses were almost as large as those observed in healthy individuals ($p < 0.01$ and $p < 0.001$ respectively). The R wave variation index obtained by summing up these two exercise and postexercise R wave shifts correlated well with the capacity to increase the PWC by training after myocardial infarction ($r = 0.66$, $p < 0.001$) (Fig. 3). Notable in this connection are the observations that neither the initial working capacity nor the maximum heart rate during the first exercise test could predict so well the subsequent result of the physical training programme ($r = 0.33$ and $r = 0.27$ respectively).

Fig. 4 displays an illustrative example concerning sequential changes of the R wave amplitude and heart rate during exercise testing in a healthy individual. The respiratory effect is great and is further augmented by the increasing work load. The height of the R wave first slightly increases and later decreases with heavier work loads. The lowest amplitude is reached immediately after exercise when resting in the supine position. Fig. 5 illustrates the "rigidity" of sequential QRS amplitude changes in a patient with very poor response to physical training after myocardial infarction.

DISCUSSION

The data obtained in the present study demonstrate that the effect of training in increasing PWC in patients with ischemic heart disease may be predicted by an index derived from the amplitude changes of the R wave which are induced by the physical exercise test. Originally Barry et al. (2) paid attention to the association between the increase of the R wave amplitude during exercise testing and the degree of ability to increase working capacity by long-term training. However in the present work this correlation was surpassed by the index of bidirectional R wave changes as compared to the initial increase of R wave alone at the start of work. The predictive power of both the initial fitness and of the initial maximum exercise heart rate turned out to be much smaller.

The mechanisms relevant for the amplitude variations observed in the QRS complex are not yet established. These may be appropriately discussed from two viewpoints: firstly in the framework of normal hemodynamic behaviour of human or animal heart and circulation during

VARIATION INDEX
OF R WAVE

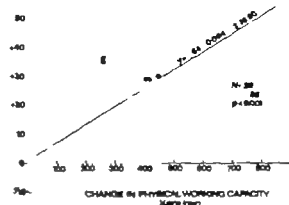


Fig. 5 Correlation between the "R" wave variation index and the subsequent change in PWC of CHD patients during the 3-month training programme.

various grades of physical exercise, and secondly in terms of the relation of these physiological variables to electrocardiographic QRS alterations observed simultaneously.

The increment of the R wave amplitude at the very initial stages of exercise may be mainly related to the transient sharp decrease of the left ventricular end-diastolic volume and to the abruptly increasing sympathetic tone taking place at this stage (19, 20, 21). That shifts in spatial QRS magnitudes are related to ventricular end-diastolic volumes has been postulated in experimental and human series (1, 16).

An even more complex set of physiological variables emerges during subsequent harder exercise in the work test. At least the following factors may be responsible for the later diminution of R wave amplitude: directional changes in ventricular preload, position of the heart inside the thoracic cage, proximity effects of the recording electrode, and hematocrit shifts.

Cardiac output is greatly increased with exercise, not only by fast heart rate but by progressively enlarging stroke volume (14). The augmented venous return consequently leads to a larger end-diastolic ventricular volume. Contrary to the increase of the R wave occurring at the very start of exercise a diminishing amplitude is now observed. The above cardiodynamic alterations related to ventricular preload may be in

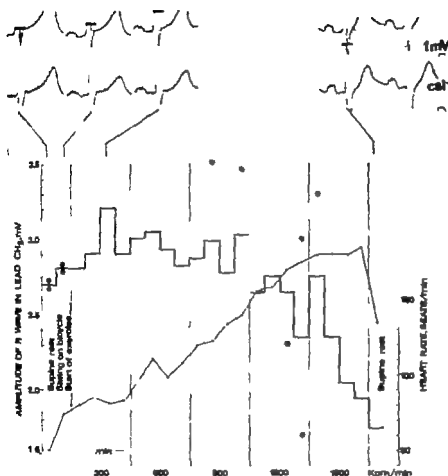


Fig 4 QRS amplitude variation during exercise testing of a healthy subject. After an initial slight asynchronisation of the R wave at the start of exercise, its height shows stepwise decreases with the progressively increasing work load. The average R wave amplitude, — the instantaneous R wave amplitudes at inspiration and at expiration, CH₃ lead was used throughout the test.

some way associated with QRS magnitude. A similar surprising decrease in R wave has been also noted when end-diastolic volume is enlarged by a pathological mechanism i.e. congestive heart failure (8). Opposite changes achieved by digitalis in these situations confirm this reasoning, although contractility alterations as such have been suggested to cause the shifts (13). The latter opinion, however would not fit with the effects seen concomitant to the increasing sympathetic

tone in heavy exercise. According to our preliminary observations, administration of a β -blocking drug, propranolol, decreases the variation of QRS amplitude during exercise. Whether this is related to change in cardiac contractility or volume still remains an open question.

A marked rotation of the QRS axis posteriorly and downward and diminution of the spatial magnitude of the QRS vector loop have been recorded after heavy anaerobic exercise (19, 11). During

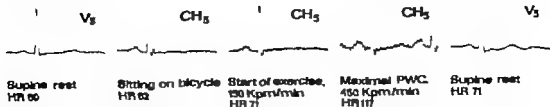


Fig 5 Prediction by exercise ECG of post effect achieved during subsequent physical training in patient

after myocardial infarction. The increase was only 113 bpm/min despite regular training during 3 months

maximum exercise the respiration rate becomes high, the excursions of the chest wall and diaphragm occur closer to the inspiration position and the residual air space increases. Thus the position of the heart is changed (3). Furthermore, another transfer impedance factor during the large chest wall excursions is the distance of the heart from the recording electrode (17). This is obvious at an extreme work level when the R wave diminution reaches its greatest degree, concomitantly with the largest oscillations caused by the respiratory movements.

Experimental raising of the hematocrit results in increased electrical resistivity of the intracavitary blood (18). The consequent reduction in "Brody effect" (4) tends to decrease radially oriented electromotive forces. Since a decrease of plasma volume is known to occur in physical exercise (12), this electrophysiological phenomenon might also contribute to R wave diminution. However hematocrit changes in central circulation during exercise are reported to be negligible (5).

In patients with angina pectoris the maximum PWC is often limited by the appearance of symptoms. Thus, in patients with angina, QRS amplitude changes induced by exercise may be less influenced by mechanical alterations related to respiration, resulting in electrode proximity effects and changes relative to the position of the heart.

In addition, an attractive possibility is that entricular volume changes remain much smaller due to either chronic or acute reduction of the entricular compliance. It is well known that stiffening of the heart muscle by gradual fibrous replacement of the muscle fibres is caused by chronic ischemia, but acute ischemia as well is

capable of causing a sudden and considerable lowering of the ventricular compliance (7). This hemodynamic parameter might then well explain the non-responsive R wave neither increasing at the start of exercise nor decreasing after exercise. In the immediate postexercise stage the excess return remains still large (6): the left ventricular end-diastolic volume is further augmented by a change to the supine position, and also by simultaneous marked reduction in the heart rate. The maximum decrease of R wave amplitude takes place at this stage (coinciding with the highest end-diastolic volume is further augmented by a does not react to the increased preload by the same increment of its diastolic volume as does heart with normal compliance. How these volume alterations are reflected in QRS magnitude is not so far understood.

The present R wave variation index may indicate how near the patient is to his maximum aerobic working capacity and may also give information about the cardiac compliance i.e. from the hemodynamic aspect of the exercise ECG. This measurement might prove to be a helpful adjunct in selection of patients for training programmes.

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A HOMOGENOUSLY INVESTIGATED HIGHLY SELECTED HYPERTENSIVE POPULATION

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Abstract A description is given of 238 hypertensive subjects examined and treated at the Hypertension Out-patient Clinic, Sahlgren Hospital. The results of the diagnostic investigations for secondary causes and organ manifestations and the therapeutic results are analyzed in the different age groups. The frequency of left ventricular hypertrophy (LVH) on ECG and X-ray rose with age, but even above 60 years of age half of these highly selected hypertensives had no signs of LVH. The percentage with enlargement of the heart also rises with age, but the rise is not so steep. Signs of renal involvement were few. The percentage abnormal serum creatinine was about 5% in all age groups, the frequency of albuminuria about 12%. In all age groups, renovascular hypertension or renal damage judged from roentgenography, I. pyelography and renal angiography were rare: active renal artery stenosis as found in less than 1%.

Two sources of information for research in the hypertensive field are available, hospital series and population studies. Both have distinct disadvantages. Hospital populations are highly selected and therefore unrepresentative of the hypertensives in the total population. Population studies often cover a limited number of cases, which means a lack of the uncommon, severe cases of hypertensive patients and finally the organ manifestations are seldom thoroughly investigated, which makes the classification into homogenous subgroups, according to the degree of organ involvement, less exact.

The hospital out-patient departments treat severe cases with a high frequency of organ manifestations, co-existing diseases and often multiple risk factors for cardiovascular diseases. This demands skilled physicians and special means for the diagnostic and therapeutic work.

The purpose of this paper is to describe the results of the routine diagnostic investigation made

on the patients referred to the Hypertension Clinic at Sahlgren's Hospital Oct. 1970-Oct. 1971. The results will later also be used for comparison with the results of the same investigative programme in the hypertensive subjects found in a multifactor primary preventive study based on random samples of the total male population (6).

MATERIAL

The patients were referred to the Hypertension Clinic at Sahlgren Hospital partly from colleagues at the hospital, partly from general practitioners or industrial physicians. As a rule all diagnostic investigations were performed on an out-patient basis and the patients were hospitalized only in case of suspicion of secondary hypertension arose.

The total number of investigated patients still under management at the Hypertension Clinic was 238. The total number of patients visiting the Hypertension Clinic during the first year (Oct. 1 1970-Oct. 1, 1971) was 280. Forty-two patients are not accounted for in this study for different reasons, e.g. they were not considered hypertensive, they did not complete the investigations or left town. The drop-out group has not been studied. The age and sex distribution is seen in Fig. 1. No attempt to analyze the sex differences was made as the number of patients in each age cohort was too small.

METHODS FOR DIAGNOSTIC INVESTIGATIONS

The diagnostic work began with two visits to the nurse for blood pressure measurements and laboratory tests and, when the doctor first saw the patient, chest X-ray and an ECG including precordial leads had also been performed. The laboratory tests routinely performed included: Hb count, ESR, serum bilirubin and alkaline phosphatase, SGOT SGPT, serum uric acid, cholesterol, triglycerides, serum electrolytes, urinary tests for proteinuria (Albumin₂) and glucosuria (Climax₂), urinary sediment and

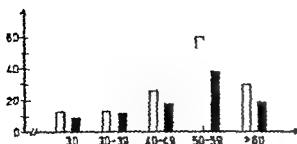
Number of
patients

Fig. 1 Age and sex distribution. □, men; ■ women.

bacterial culture, and test for urinary osmolality after 13 hours' thirst.

Most data are collected from the investigations performed at the Hypertension Clinic, but in some cases laboratory tests from patients under care in some of the medical departments have been used if the tests were not more than 3 months old or in the case of X-ray of the heart, 6 months old.

All BPs were taken by the same nurse in both arms after 5 min in the recumbent position and after 1 min in the standing position. A mercurial manometer was used and the arm cuff was 12.5 cm broad and 4 cm long.

The routine chest X-ray was examined by different roentgenologists for configuration and volume of the heart related to BSA.

The criterion for left ventricular hypertrophy (LVH) was the subjective finding of more rounded or prominent left ventricle.

The ECG was analysed by the investigator. Three of the following four criteria were necessary for the diagnosis of LVH: 1) Negative T wave in lead VL (or in VF in subjects with atypical heart positions). 2) Sum of r in lead Cr and R in lead Cr > 35 mm. 3) Intraventricular activation time > 0.05 sec. 4) Flattened or

Per cent

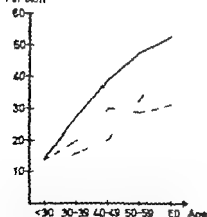


Fig. 2. Hypertensive heart manifestations. — LVH on ECG; --- LVH on X-ray; ··· heart enlargement on X-ray.

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Per cent

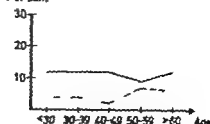


Fig. 3 Hypertensive kidney manifestations. Serum creatinine > 1.4 — albuminuria.

negative T waves in leads Cr and Cf. Patients with LBBB, atrial fibrillation or signs of coronary artery disease have not been included in the analysis. The data are referred to as missing data.

The final examinations were performed by the physician examining the patient at the Hypertension Out-patient Clinic. During the time of the study six physicians are working at the Hypertension Clinic. The subdivision into four categories was made according to Keith-Wagener-Barker (1), but stages I and II were brought together into one group in an attempt to diminish the observer variation.

The serum creatinine was analysed with an auto-analyser. Urinary sediment of fresh voided urine was microscopically examined at once, albuminuria and glucose were established by strip methods (Abbot's T-Chemstrips).

Further investigations for renal hypertension were performed almost as a routine in younger patients (below 40 years), in the older age groups only when a clinical suspicion of renal involvement existed or when good control of BP was difficult to achieve. Isotope renography was used as a screening test and, if necessary the investigation was carried on with I. pyelography (IVP) or renal arteriography performed by the renal Schölkopf technique. When a routine urinary test gave rise to suspicion of renal disease the IVP was predominantly performed. No rapid sequence IVP was done. The isotope renography was performed as described by Amel et al. (1).

RESULTS AND DISCUSSION

Organ manifestations

The severity of the hypertensive disease, described by the organ manifestations, is shown in Fig. 1 and 3 and Table I.

Heart

Fig. 2 shows the hypertensive heart manifestations. LVH percentage on ECG was 9% below the age of 30 then steadily rising with advanced age but even above 60 years of age half of these highly selected hypertensives had no signs of LVH.

Table I *Fundl according to Keith-Wagener Barker Distribution (in %) of FH groups in different age groups*

	<30 (-22)	30-39 (n=25)	40-49 (n=44)	50-59 (-98)	>60 (-49)
Normal	54	36	23	26	16
FH I-II	46	52	61	50	62
FH III	0	4	4	2	2
FH IV	0	0	2	2	0
No data	0	4	8	14	20

on ECG. The same holds for the X-ray of the chest, although the figures were somewhat higher. The heart volume related to BSA also rises with age, but the rise is not as steep as for LVH. The slopes for rising percentage of LVH on ECG and X-ray are much steeper than the slope for the rise in percentage of heart enlargement. There is 14% heart enlargement already below the age of 30 and then the percentage only rises moderately which may indicate that those who develop enlargement of the heart do so at an early stage. Those who developed severe heart enlargement (above 600 ml/m² BSA) did so after the age of 50, but some of these patients had experienced myocardial infarctions that could account for their heart damage. When these figures for hypertensive heart disease (HHD) are compared to population studies (2, 5), the frequency for HHD seems to be about the same in our highly selected material as in non-selected population studies. In the younger age groups the percentage of HHD was even lower. These somewhat unexpected facts might be explained by slight differences in criteria for HHD on both ECG and X-ray but many other explanations (or speculations) are possible.

The percentage of LVH on ECG rises with age, starting with only 9% below 30 years of age, rising to 49% above 60 years of age. The frequency of LVH, judged from a chest X-ray rises with age in a similar fashion as for ECG. The same holds for heart enlargement, but the rise is not so steep. The percentage of missing data was negligible (<8%).

Fundl

The eyeground changes are demonstrated in Table I. FH I-II predominate throughout the age groups.

Table II *Percentage of results of urinary sediment (microscopic pyuria and/or hematuria), urinary bacterial culture and tests for urinary glucose with rising age*

Figures within parentheses show the number of patients with an abnormal finding in relation to the total number examined in that age group

	<30 (-22)	30-39 (n=25)	40-49 (n=44)	50-59 (n=98)	>60 (-49)
Sediment					
Normal	91	100	81 (97)	92 (95)	84 (90)
Pyuria	0	0	2 (3)	3 (4)	4 (5)
Hematuria	0	0	1 (1)	4 (5)	4 (5)
No data	9	0	5	4	6
Urinary bacterial culture					
Negative	82	88	91	92	89
Positive	0	0	2 (2)	2 (2)	2 (7)
No data	18	8	5	6	9
Urinary glucose					
Negative	77	88	91	92	90
Positive	0	4 (5)	6	2 (2)	2 (7)
No data	23	8	9	6	8

Kidney

Fig. 3 shows the kidney manifestations. The missing data were few (below 10% in all age groups). The percentage of abnormal serum creatinine is stable around 4 throughout the age groups. Only one patient had creatinine above 1.9 mg/100 ml. This patient had no history of pyelonephritis or glomerulonephritis, no renal artery stenosis on angiography but bilateral, parenchymal reduction, and this renal damage was thought to be due to the hypertensive disease.

Also the percentage of proteinuria was rather steady around 12% perhaps indicating that those who develop renal involvement do so at an early age. The higher percentage of albuminuria than of abnormal serum creatinine also indicates that a semiquantitative test for albuminuria is a more sensitive method than serum creatinine in revealing renal damage due to hypertension.

Table II shows the results of urinary sediment, urinary bacterial culture and tests for urinary glucose. There were few missing data and few abnormal findings. Nine patients with glucose in the urine at the initial diagnostic investigation were all known to have diabetes.

Abnormal urinary sediment was very rare throughout the age groups. Pyuria and hematuria

Table III Percentage of normal results, abnormal types of curves and missing data of isotope renography in different age groups

Figures within parentheses show the number of patients with abnormal type of curve

	<30 (n=22)	30-39 (n=25)	40-49 (n=44)	50-59 (n=98)	>60 (n=49)
<i>After 12 hours thirst</i>					
Normal	46	32	40	38	21
Type C curve	14 (3)	8 (2)	11 (5)	2 (2)	4 (2)
Type B curve	14 (3)	8 (2)	11 (5)	2 (2)	2 (1)
Type A curve	0	4 (1)	7 (3)	5 (5)	0
No data	28	48	40	53	74
<i>After hydration</i>					
Normal	72	44	51	41	25
Type C curve	0	0	2 (1)	1 (1)	0
Type B curve	0	4 (1)	0	0	2 (1)
Type A curve	0	4 (1)	7 (3)	5 (5)	0
No data	28	48	40	53	74

were highest in the oldest age group about 5% but this might also be due to other factors than hypertension. Positive urine cultures for bacteria were even rarer than abnormal sediment, about 2% positive cultures were only found in the older age groups and it seems unreasonable to ascribe these findings to the hypertension.

Evaluation of renal causes of hypertension

The results of further investigations for renovascular or renal parenchymal hypertension are shown in Table III. There is a high proportion of missing data for renography IVP and renal arteriography which diminishes the conclusions that can be drawn.

Table III shows the findings from the isotope renography after 12 hours thirst and after mild hydration (10 ml/kg b.wt). There were 14 renograms of the type C that is, with signs of slowed excretion on one side (4), after 12 hours thirst, but after hydration only 2 remained abnormal. One of these two patients had an ampullary renal pelvis on the same side as the abnormal renogram and the other patient had a normal IVP. There were 9 renograms of the type B i.e. with signs of slowed parenchymal uptake on one side in dehydration; only two remained abnormal after hydration. Both these patients had a renal artery stenosis. Nine renograms of type A, i.e. with signs of severe parenchymal reduction in dehydrated

condition remained abnormal after hydration, reflecting unilateral renal hypoplasia or end stage of unilateral chronic pyelonephritis (IVP) in seven cases and status post nephrectomy in two.

The renogram is judged abnormal only if it is abnormal both in dehydrated and hydrated condition. With these criteria only 13 patients (5% of the total number of cases) had abnormal renograms. Nine of these 13 (4%) had renal changes that were judged to be the probable cause of their hypertension: 2 had main renal artery stenosis, 7 unilateral advanced parenchymal damage due to chronic pyelonephritis or renal hypoplasia.

IVPs were performed in 64% of those under 30 years of age; the percentage fell with age to 23% for those above 60. Renal arteriograms were performed in 35% of those under 30 years of age, but in older age groups only a very limited number were examined. Only two patients were shown to have an "active" renal artery stenosis (with significant renin production) which could be submitted to reconstructive surgery.

The figures for renovascular or renoparenchymal causes of hypertension might be underestimated because the routes of selection in these cases might lead towards the Department of Nephrology and those hypertensive cases are not included in this material.

Table IV Distribution (in %) of drugs and blood pressure control in the different age groups

	<30 (n=22)	30-39 (n=25)	40-49 (n=44)	50-59 (n=98)	>60 (n=49)
<i>Drugs</i>					
Propranolol	23	24	30	23	16
Alprenolol	23	2	11	7	13
Hydralazine + propranolol					
alprenolol	14	4	34	43	27
Saluretics	9	20	34	34	49
Other	5	28	25	29	37
No treatment	30	23	11	7	6
<i>BP control^a</i>					
Good ($<160/ <90$)	41	36	4	24	20
Acceptable ($\geq 160/ \geq 90$ - $<175/ <110$)	58	52	65	73	77
Poor ($\geq 175/ \geq 110$)	0	12	11	23	37

^a Judged from the latest recorded BP (right arm, lies after 5 min rest)

Treatment

The hypotensive treatment used and its outcome are shown in Table IV. At the time of this study 30% below 30 and 25% 30-39 years of age had no treatment. The percentage of patients treated with saluretics increased with age. Above 60 years of age almost half of the patients received saluretics. β -blocking agents (alprenolol or propranolol alone or in combination) predominated even if the percentage diminished somewhat with age at the same time as the combined therapy with hydralazine and β -blockers increased. The criteria for good, acceptable and poor BP control are also seen in Table IV.

The latest recorded BP has been used for judgement of BP control. Up to the age of 60 there is a rising proportion of poorly controlled patients. In the age group 50-59 years 23% have BP above 175/110 despite treatment, which shows that even in skilled hands good BP control is not easily achieved. One explanation of the poor therapeutic results in this material might be that the most common cause for referral was "poor BP control" and our material seems to contain a large proportion of patients who were resistant to any form of antihypertensive treatment.

The reason for the high proportion of medically untreated cases under 40 years of age is the study that was being made at that time on training of hypertensive subjects.

GENERAL COMMENTS

Special out-patient hypertension clinics have been established in many places in an effort to make the management of hypertensive patients more continuous and effective. A thorough investigation for organ involvement and co-existing diseases is the basis for homogenous subgrouping, on which the evaluation of the therapeutic results must rest. The results of the investigations reported in this paper will be used for classification into homogenous subgroups and the patients will be followed up at 1 year intervals to see in which subgroups antihypertensive treatment gives the best results.

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PLASMA VOLUME AND EXTRACELLULAR FLUID VOLUME IN ESSENTIAL HYPERTENSION

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Abstract Plasma volume (PV), extracellular fluid volume (ECV) and the ratio of plasma volume to interstitial fluid volume (PV/IF), have been determined in 35 patients (18 males and 17 females) with untreated essential hypertension of medium severity without cardiac or renal complications, and in a similar number of normotensive individuals. As regards age, height, weight and sex, the normotensives were fully comparable with the patients of the hypertensive group. In hypertensive men the plasma volume and PV/IF were significantly lower than in normotensive men ($p < 0.001$ and $p < 0.005$ respectively). The ECV in hypertensive patients and in normal individuals did not differ significantly. In women the same trend was observed, but the differences were not significant. The results are compared with those from previous studies. Our findings agree with the most recent and best controlled investigations. It seems reasonable to explain the changes observed by increased venous tone and increased capillary hydrostatic pressure.

Numerous studies have been made of plasma volume (PV) and extracellular fluid volume (ECV) in patients suffering from essential hypertension. The results have led to contradictory opinions. Whereas some investigators found PV to be reduced (13, 21, 23, 25, 27), others found normal values (7, 13, 26, 28). Similar contradictory results have been found as regards ECV. Several investigators reported normal values (8, 14, 25) whereas others were of the opinion that ECV is increased in essential hypertension (19, 22, 26). Furthermore it has been maintained that there is an abnormal distribution between the intravascular and the extravascular components of the ECV (23) but only few simultaneous studies of PV and ECV are available.

The object of the present study was to compare PV, ECV and PV/IF (interstitial fluid volume) in patients with essential hypertension of medium severity without cardiac or renal com-

plications, with the findings in a normotensive control group.

MATERIAL AND METHODS

The subjects of this study are 18 males and 17 females with untreated essential hypertension, and the control groups comprised the same number of males and females. Age, height, weight, BSA and BP are presented in Table I.

All patients with essential hypertension are hospitalized, and those causes of hypertension were excluded through an examination programme including serum electrolytes, urinary excretion of catecholamines, creatinine clearance, urography and isotope roentgenography. All had normal cardiac volume, determined by chest X-ray, none had past history or present evidence of cardiac failure. ECG showed no signs of left ventricular strain. Renal function was normal in all the patients, as assessed by creatinine clearance. Weight did not differ by more than 10% from that normal for the age of any of the patients. Twenty-eight patients presented normal changes corresponding to grade 2 (Keith-Wagner) four males and one female had grade 3 and one male and two females grade 1 changes. Antihypertensive therapy was discontinued in all cases for at least four weeks before the investigation.

The controls were patients who had been referred for diseases which would not influence PV or ECV. None of the controls received drugs which might influence the above mentioned parameters.

Both the hypertensive patients and the controls are hospitalized at the time of the investigation, but they were ambulatory throughout the daytime and were kept on normal hospital diet.

Prior to the investigation, each subject started at 8 a.m., all the patients had been fasting, had had nothing to drink and had been confined to bed for 10 hours. The investigation was made with the patient in the supine position. PV (T 1824) and ECV (^{51}Cr space) were determined as previously described (17, 18). In the patients as well as in the controls PV and ECV were determined simultaneously. PV/IF was calculated (IF defined as ECV-PV).

Statistical calculations were made employing Student's *t*-test for comparison of averages. A difference was regarded as significant if the *p*-value was less than 0.05.

Table 1 PV, ECV and PV/IF in normotensive men and women and in men and women with essential hypertension

		BP (mmHg)										
		Height (cm)	Weight (kg)	BSA (m ²)	Age (y)	Systo- lic	Diasto- lic	PV (ml)	PV/m ² (ml)	ECV (ml)	ECV/m ² (ml)	PV/IF
Men												
I Normo- tensive	Mean	174.5	73.1	1.87	45.8	131	79	3 540	1 891	17 392	9 238	0.257
	S.D.	5.6	8.9	0.12	12.3	16	7	389	187	2 082	787	0.041
	S.E.M.	1.3	2.1	0.03	2.9	4	2	92	44	504	191	0.01
	n	18	18	18	18	18	18	18	18	17	17	17
II Hyper- tensive	Mean	175.3	75.0	1.89	49.0	183	119	3 176	1 682	17 535	9 207	0.223
	S.D.	4.3	9.4	0.13	8.8	17	12	393	133	1 946	701	0.025
	S.E.M.	1.0	2.2	0.03	2.1	5	3	92	31	502	181	0.008
	n	18	18	18	18	18	18	18	18	15	15	15
Δ II-I								-364	-209	+143	-31	-0.034
t								2.7913	3.8699			3.4090
p								<0.01	<0.001	n.s.	n.s.	<0.005
Women												
I Normo- tensive	Mean	162.4	57.7	1.61	48.0	136	84	2 997	1 617	12 950	8 043	0.254
	S.D.	5.18	8.27	0.11	13.4	15	5	386	175	1 603	751	0.033
	S.E.M.	1.26	2.00	0.03	3.3	4	2	93	42	388	182	0.008
	n	17	17	17	17	17	17	17	17	17	17	17
II Hyper- tensive	Mean	161.1	60.2	1.63	48.5	185	114	2 530	1 546	13 251	8 077	0.232
	S.D.	5.61	8.45	0.12	11.8	20	8	389	182	1 958	714	0.027
	S.E.M.	1.36	2.04	0.03	2.9	5	2	94	99	565	206	0.008
	n	17	17	17	17	17	17	17	17	12	12	12
Δ II-I								-67	-71	+301	-6	-0.022
t										1.225		1.908
p								n.s.	<0.3	n.s.	n.s.	<0.1

RESULTS

As will be seen from Table 1 the patients and controls were fully comparable as regards age, height, weight and BSA.

In hypertensive males (Table I) the PV was significantly lower compared with the normotensive subjects, 3 176 ml against 3 540 ml, $p < 0.01$ or 1 682 ml/m² against 1 891 ml/m², $p < 0.001$. ECV was practically identical in the two groups. PV/IF was significantly lower in the hypertensive patients, 0.223 against 0.257, $p < 0.005$.

PV and ECV in females with essential hypertension were not significantly different from the results found in the controls (Table I). The average PV/m² BSA however was lower in the hypertensive group 1 546 ml against 1 617 ml and also the PV/IF was found to be somewhat lower in the hypertensive group, although the difference was not significant ($0.05 < p < 0.1$).

Neither in males nor in females was it possible

to demonstrate any correlation between PV and diastolic BP or mean BP. In males no correlation was found between PV and the known duration of hypertension whereas in females there was a negative correlation between PV and the duration in months of the hypertension ($r = -0.4944$, $p < 0.05$).

DISCUSSION

The contradictory results of studies into body fluid compartments in hypertension reported in the literature are, presumably to a certain extent determined by the number of variables which are not sufficiently taken into account when composing the control groups, e.g. sex, height and weight. On the other hand, differences in the age are of no greater importance. It has been shown that within very wide limits PV and ECV are independent of age (3, 5, 23). Furthermore, complicating factors such as unrecognized moderate cardiac failure may play a role. Several factors

gators have considered patients with arterial hypertension as a homogenous group (7, 16, 28). Since some studies seem to indicate that PV and ECV differ in various types of hypertension (14, 23, 4) it is necessary to define strictly the type of hypertension in the patients examined.

Our results show that PV is reduced in males with essential hypertension. With a few exceptions (13, 29) this is in agreement with more recent and well controlled studies (15, 23, 25). We were unable to demonstrate a significant difference in females, although the results seem to indicate a reduced PV. Most likely changes in the fluid balance within the menstrual cycle may contribute, and in order to avoid this possible source of error females have been excluded in many studies. In one study reduced PV was found in females with essential hypertension (24). A few investigators (15, 25) have found negative correlation between PV and diastolic BP in comprehensive series, 36 and 37 males, respectively. We were unable to verify this finding, possibly because of the limited number of patients in our series. In a more recent work Tarazi et al. (24) however could demonstrate this correlation only in 8 patients with an inappropriate plasma volume expansion; were excluded from a series comprising 55 hypertensive males, and in 17 females such a correlation was not found. None of our patients had increased PV, the highest value being 1866 ml/m².

Some investigators have found negative correlation between PV and the total peripheral resistance (1, 2, 10, 15). Furthermore, Birkenhäger et al. (2, 3) and Julius et al. (15) found a positive relationship between PV and cardiac output. Because there is considerable evidence that cardiac output falls and peripheral resistance increases during the course of essential hypertension (1, 4, 9, 16), it would not be surprising if an inverse correlation was found between PV and the known duration of hypertension. We were unable to find such a relationship in males, and in females it was only just significant at the 5% level. A contributory factor might be that many of the patients examined had had hypertension only for a short time: 5 cases, both men and women, for less than one year.

Hence it seems to be proved that PV is reduced in uncomplicated essential hypertension in medium and severe cases (1). Conversely the conditions in

mild and labile hypertension are less clear. Tarazi et al. (24, 25) found that reduced PV can only be demonstrated if the diastolic BP exceeds 105 mmHg, whereas Julius et al. (15) found reduced PV also in cases of labile hypertension, and Moltzahn et al. (20) had the same results even in a limited series (10 males).

In the present study no significant differences could be observed between ECV in normal individuals and in patients with essential hypertension. This is in agreement with some investigations (8, 14, 13, 29), but does not agree with results of others (12, 19, 22, 16). The contradictory results must be due to a certain extent to differences in the selection of patients and the classification of hypertension. As we knew it, some investigators have not distinguished between essential hypertension and other types of the disease (17, 6). In one study there was an appreciable preponderance of patients with renal parenchymal disease (19). In another study (21) ECV was determined only in patients who had increased total exchangeable sodium, and 7 of the 10 patients had malignant hypertension. The study performed by Groffman and Shapiro (12) comprised patients with minimal cardiac impairment. However, Hollander et al. (14) have found that ECV is increased in malignant hypertension and in cases complicated by even slight cardiac failure. The studies which agree with our results are those relating to patients with well defined essential hypertension and comparable controls.

Although our study shows that ECV is normal in cases of uncomplicated hypertension we find that, as compared with normotensive individuals, there is a changed distribution between its intravascular and its interstitial components, which will be seen from the significantly lower PV/IF. The same changes in ratio were found by Tarazi et al. (23).

The reduced PV must be accompanied by a slightly reduced venous capacity. Since the total ECV remains unchanged, and the PV/IF is lower, this reduction cannot be expected to result from increased renal excretion of fluid, but from displacement of plasma from the intravascular to the extravascular space. Therefore it must be supposed that the venous tone and the capillary hydrostatic pressure are increased in essential hypertension, resulting in the changed equilibrium between PV and IF (6, 11, 30).

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GLUCOSE TOLERANCE, INSULIN RELEASE AND LIPOPROTEIN PATTERN
IN PATIENTS AFTER MYOCARDIAL INFARCTION

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Abstract. Of 57 men examined, on average, 26 months after their first myocardial infarction 47% had an abnormal glucose tolerance test compared with 10% of 20 healthy controls. The abnormal glucose tolerance was demonstrated in 73% of patients with type IV and 53% of those with normal lipoprotein pattern, but only in 29% of type II patients. The fasting level of immunoreactive insulin and the values from 20-120 min after the glucose load were significantly elevated among patients. The hyperinsulinemia was most evident among the type IV patients (significantly different also from the other patient groups) while the type II patients had the least pronounced insulin elevation. Hyperinsulinemia as possible "risk factor" for development of atherosclerotic disease is discussed.

In previous studies of the lipoprotein (LP) pattern of patients surviving myocardial infarction (9, 26) we found slightly impaired oral glucose tolerance in 68% of the patients with a Fredrickson's type IV pattern.

It is well known that atherosclerotic complications occur often in diabetic patients. During the last years the relationship between atherosclerotic disease and abnormal carbohydrate metabolism even in patients without recognized diabetes mellitus has been discussed. We have therefore considered it of interest to reexamine our patients for glucose tolerance and insulin release and to correlate eventual abnormalities to the LP

As controls we have examined 20 men below the age of 70, without any known disease recruited through local Rotary Club. Their average age was 55 years, compared with 58 years for the patients ($p > 0.10$). One of the controls had mild untreated hypertension. Seventeen had normal and 3 type II LP. Thirty-two percent of the patients and 25% of the controls are 10-25 over weight (Table I).

The LP of the 57 patients, and of one who was withdrawn from this study because of circulatory collapse of unknown cause 15 min after glucose infusion, was discussed in previous publication (26), in which the patient material and methods for classification of the LP were described in detail. The patients are the survivors among the male patients under 70 years without renal, liver or endocrine disease except diabetes mellitus, treated in the hospital for their first myocardial infarction in 1967-69.

The patients were considered to have normal LP with cholesterol < 325 mg/100 ml = 8.4 mmol/l and triglycerides < 150 mg/100 ml = 1.65 mmol/l in three examinations during the first three months and again about two years after the infarction.

The LP of patients with only one cholesterol estimation, 325-350 mg/100 ml, or one triglyceride estimation, 150-200 mg/100 ml, have been considered indefinite.

The hyperlipidemic patients have been referred to type II or type IV according to the lipoprotein electrophoresis and serum lipid values.

The mean cholesterol and triglyceride values for patients after two years, and for the controls, are given in Table I.

The glucose tolerance and insulin release in response to glucose have been examined in patients and controls after infusion of 25% glucose, 0.5 g/kg b.wt during 5 min. B-glucose and p-creatinin were determined in venous samples taken in the fasting state and 5, 10, 20, 30, 45, 60, 90 and 120 min after the start of infusion.

The patients and controls were on their normal diet. They were examined as outpatients after overnight fasting. During the glucose tolerance test they were recumbent.

Glucose has been determined by the o-toluidine method according to Hinkinen (13), insulin in triplicate as immunoreactive insulin (IRI) as hyperoxidized plasma by the double antibody method according to Hales and Randle

MATERIAL AND METHODS

Fifty-seven male patients have been studied, on average, 26 (16-32) months after their first myocardial infarction. Four of them had mild diabetes mellitus treated with diet and chlorpropamide.

Seventeen (30%) had normal LP pattern, 17 (30%) type II, 8 of whom type II with pre- β (type IIb), 15 (26%) type IV and 8 (14%) indefinite hyperlipoproteinaemia.

Table I. Number of controls and patients with different lipoprotein pattern, diabetes mellitus and overweight age and serum lipids (mean \pm S.D.) 16-32 months after myocardial infarction (percentages given within parentheses)

	LP pattern					
	Controls 20	Patients 57	Normal 17 (30)	Type IV 15 (26)	Type II 17 (30)	Indefinite 8 (14)
Diabetes mellitus	0	4		2	2	
10-25% overweight	5 (25)	18 (32)	3	7	6	
Age (y)	55 \pm 8	59 \pm 10	59 \pm 10	60 \pm 10	58 \pm 10	61 \pm 10
Cholesterol (mg/100 ml)	295 \pm 57	336 \pm 66	290 \pm 32	331 \pm 39	398 \pm 78	315 \pm 34
Triglycerides (mg/100 ml)	76 \pm 34	126 \pm 81	79 \pm 32	233 \pm 90	105 \pm 41	95 \pm 31

Table II. Glucose tolerance in normals and patients with ischemic cardiovascular disease

		Glucose tolerance (%)			Mean <i>k</i> -value	Mean age (y)
<i>N</i>		Diabetic	Borderline	Normal		
Normals						
Wahlberg (34)	200	4	10	86	1.53	57
Others	443	5	9	86	1.54	
Present study	20	0	10	90	1.94	55
Ischemic disease						
Passchyl (21)	178	29	28	43	1.14	59
Wahlberg (34) and 6 others	414		61	39	1.23 ^a	54 ^a
Present study	57	23	24	54	1.29 ^b	59

5 studies, ^a $p < 0.01$ vs. controls.

(11), with reagents from Radiochemical Centre Amer sham.

To evaluate the glucose tolerance curves the *k*-values (percentual reduction of blood glucose/min from 15-20 to 60 min after the start of the infusion) have been calculated. According to Wahlberg (34) $k > 1.10$ is considered normal, $k < 0.91$ diabetic and k 0.91-1.10 borderline.

The "insulinogenic index" (29) has been calculated for the first 20 min by dividing the area under the insulin curve above fasting level by the corresponding glucose rise. Calculations of statistical significance have been performed with the Student's *t*-test.

RESULTS

Table III. Glucose tolerance in patients with different lipoprotein pattern

		Glucose tolerance (no. of pati.)			Mean <i>k</i> -value
LP pattern	A	Diabetic (diab. mell.)	Border- line	Normal	
Normal	17	3	6	8 (47%)	1.24
Type IV	15	6 ^a	5	4 (27%)	1.00 ^b
Type II	17	4	1	12 (71%)	1.48 ^a
Indefinite	8		2	6 (75%)	1.56

2 of which diabetes mellitus

^a $p < 0.01$ vs. controls.

^b $p < 0.05$ vs. controls. When excluding 2 diabetics mean $k = 1.37$ $p > 0.10$.

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Twenty-seven of 57 patients (47%) had an abnormal glucose tolerance (borderline or diabetic *k* value) two years after their first myocardial infarction, compared with two (10%) of 20 healthy controls (Table II). The mean *k*-value for the patients is 1.29 for the controls 1.94 ($p < 0.01$).

Seventy-three per cent of patients with type IV LP and 53% of those with normal serum lipids had an abnormal glucose tolerance, but only 29% of type II patients (Table III). The mean age is the same 58 years, for patients with normal and abnormal glucose tolerance. The mean *k* values of patients with normal lipids and type IV differ significantly from the controls ($p < 0.01$). The *k*

Table IV Insulinogenic index/30 min and plasma insulin (mean \pm S.D., μ U/ml) in controls and patients during *iv* glucose tolerance test

	Controls (n=20)	Patients (n=57)	Lipoprotein pattern					Indefinite (n=8)
			Normals (n=17)	Type IV (n=15)	Type II (n=17)	Type II (n=9)	Type IIb (n=8)	
Insulinogenic index/30 min	0.19 \pm 0.15	0.18 \pm 0.14	0.14 \pm 0.10	0.16 \pm 0.13	0.23 \pm 0.20	0.28	0.16	0.16 \pm 0.06
Insulin								
0'	14.3 \pm 1.6	22.8 \pm 11	18.8 \pm 5.6 ^a	33.7 \pm 16 ^a	38.8 \pm 3.0 ^a	20.0	17.4	15.3 \pm 2.4
5'	68 \pm 43	58 \pm 35	57 \pm 29	56 \pm 30	73 \pm 43	83	57	48 \pm 16
10'	48 \pm 31	53 \pm 31	46 \pm 22	46 \pm 30	58 \pm 42	73	51	41 \pm 12
20'	34 \pm 22	53 \pm 29 ^a	41 \pm 18	70 \pm 32 ^a	53 \pm 35	62	43	42 \pm 14
30'	32 \pm 17	49 \pm 24 ^a	40 \pm 16	66 \pm 29 ^a	45 \pm 19	51	38	44 \pm 15
45'	28 \pm 13	48 \pm 26 ^a	41 \pm 15 ^a	69 \pm 34 ^a	38 \pm 19	41	34	48 \pm 22 ^a
60'	23 \pm 7.5	45 \pm 25 ^a	38 \pm 12 ^a	67 \pm 34 ^a	33 \pm 15 ^a	37	30	45 \pm 16 ^a
90'	17 \pm 3.0	34 \pm 18 ^a	30 \pm 9.2 ^a	51 \pm 25 ^a	28 \pm 10 ^a	27	25	26 \pm 7.8 ^a
120'	16 \pm 1.9	28 \pm 17 ^a	26 \pm 10 ^a	42 \pm 25 ^a	20 \pm 8.1	22	19	1 \pm 6.0 ^a

^a $p < 0.01$ vs. controls. ^b $p < 0.05$ controls.

value of the type II patients is only significantly different when the two diabetics with type II pattern are included ($p < 0.05$).

The fasting levels of IRI and the values from 20 min and afterwards following *iv* glucose were significantly higher among patients than controls ($p < 0.05$ 20–30' $p < 0.01$ after 45') (Table IV Fig. 1).

When the insulin values are correlated to the LP (Table IV Fig. 2) the type IV patients deviate most from the controls, with significantly elevated mean insulin levels ($p < 0.01$) from 20 min after start of glucose infusion and a definitely elevated fasting insulin concentration. But the same pattern of insulin release, significantly different from the controls, although less pronounced, is also

present in patients with a normal LP. The type II patients differ from the controls in fasting and 60–90 min insulin values ($p < 0.01$). The insulinogenic index/30 min does not differ significantly for any group (Table IV).

DISCUSSION

The *iv* glucose tolerance values in our controls and patients are given in Table II. The Table also reports the results of other studies using the same methods of examination. The results among controls are compared to those published by Wahlberg (34), who examined his own control group and reviewed the literature for studies of the *iv* glucose tolerance in subjects without

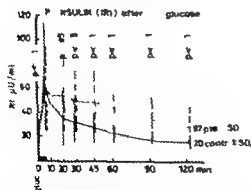


Fig. 1 Plasma insulin during 120 min after an *iv* glucose load in controls and patients after myocardial infarction (mean \pm S.D.).

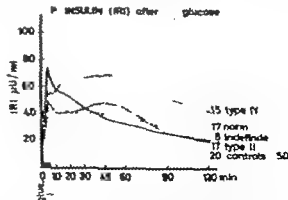


Fig. 2 Plasma insulin in controls (mean \pm S.D.) (shaded area) and patients (mean) with different LP during 120 min after an *iv* glucose load.

diabetes mellitus or cardiovascular disease. As in the present study Paasikivi (21) examined survivors from a first myocardial infarction and he has reviewed Wahlberg's (34) and six other studies (3, 7, 12, 20, 25, 28) of the i.v. glucose tolerance test in patients with ischemic cardiovascular disease. Our results correspond well to those reported above. Wahlberg, in a review of the literature, also found a remarkable agreement between i.v. and oral glucose tolerance in 590 patients with ischemic disease examined after an oral glucose load. Thus there is extensive evidence that an impaired glucose tolerance, chemical or latent diabetes mellitus, often occurs in patients with ischemic cardiovascular disease.

Seltzer et al. (29) and Cerami and Luft (6) after a more massive glucose load, postulated that a delay in insulin release after glucose is characteristic of mild diabetes and the prediabetic state. Reaven and Miller have demonstrated this delay in more pronounced glucose intolerance (23) but find an elevated insulin release at all times, i.e. hyperinsulinism after an oral glucose load in patients with chemical diabetes, with an only slightly impaired glucose tolerance. The insulin release pattern after i.v. glucose was the same as after oral glucose but the differences were smaller and not statistically significant (24). Hyperinsulinism after oral glucose has also been demonstrated by Jackson et al. (14) in potential diabetics.

In order to study whether there is a delay in insulin release we have evaluated the "early" insulin release of our patients by calculating the insulinogenic index for the first 30 min of the glucose tolerance test. We find a great variation in results and no significant differences between controls and any group of patients. Thus we cannot prove a smaller insulin release in our patients than in the controls during the first 30 min.

However the late insulin release and the fasting insulin level are significantly elevated in our patients. This pattern of insulin release resembles the findings of Reaven et al. in chemical diabetes mellitus. A similar hyperinsulinism has been demonstrated in several investigations of small groups of patients with ischemic heart disease or peripheral vascular disease (7, 16, 17, 19, 20, 22, 30, 33). In most of the investigations the hyperinsulinism had been provoked by an oral glucose load, which produces a higher insulin release than a similar i.v. dose of glucose (29).

Nikkilä et al. (20) and Malherbe et al. (19) demonstrated hyperinsulinism after oral but not after i.v. glucose. The patients of Lebovitz et al. (17) with acute and of Christiansen et al. (7) with previous myocardial infarction had significant hyperinsulinism after an i.v. glucose load. Several authors discuss the possibility that the elevated plasma insulin per se, not the hyperglycemia, may be an etiological factor for development of atheromatosis (15, 18, 19, 27, 30, 31, 32, 33). At present it can only be stated that hyperinsulinism seems to occur often in patients with ischemic disease.

Some studies have revealed a connection between serum lipids and glucose intolerance or hyperinsulinism (1, 2, 8, 10). Albrink and Davidson (2, 8) suggest that the elevation of plasma triglycerides represents a stage in the development of maturity onset diabetes or a diabetes-like state. Several other authors have found no definite relationship when correlating glucose tolerance to serum lipids in patients with ischemic cardiovascular disease (5, 7, 12, 20). The incidence of abnormal oral glucose tolerance in another type II and type IV hyperlipoproteinemias is 15 and 60% respectively (12).

Among our patients the lowered glucose tolerance and elevated plasma insulin levels are most pronounced in patients with a type IV lipoprotein pattern, i.e. patients with mainly elevated triglycerides. The mean k value in this group is in the borderline range to diabetes mellitus. The insulin levels, fasting and in the period after 20 min, are definitely elevated. Only 4 of the 15 patients have a normal glucose tolerance. The same tendency is also demonstrated among patients with normal lipoproteins. The type II patients and those with indefinite LP pattern (most of whom have slightly elevated cholesterol values) have a more normal glucose tolerance and insulin release. When comparing the patient groups, the type IV patients have significantly higher insulin values than the patients with the other lipoprotein patterns.

In our patients the plasma insulin level is most elevated in the patient groups where the recognized "risk factor" of elevated serum cholesterol is less pronounced, i.e. with type IV LP or when there is no definite hyperlipoproteinemia. In the Stockholm study (4) elevated serum triglycerides are also documented as a "risk factor".

However, hypertriglyceridemia may occur secondarily to a deranged carbohydrate metabolism.

ACKNOWLEDGEMENTS

This study is part of an investigation supported by grants from the Norwegian Council for Cardiovascular Diseases and the Scandinavian Insulin Fund.

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INSULIN CONCENTRATION IN PORTAL AND PERIPHERAL VENOUS BLOOD AFTER ORAL GLUCOSE IN HUMAN PANCREATITIS

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Abstract. Insulin and glucose concentrations in the portal and in the peripheral venous blood have been determined in connection with oral glucose loads in 1 patients with acute or relapsing acute pancreatitis. A low insulin increase in the peripheral venous blood was not invariably accompanied by poor insulin increase in the portal blood, but was sometimes due to substantial reduction of the insulin level between the portal and the peripheral venous blood. Moreover the fractional reduction of insulin between the portal and the peripheral venous blood tended to be higher at high than at low portal insulin level. The blood glucose concentration, however, seemed to be without influence upon the relation between the portal and the peripheral venous insulin concentrations.

the peripheral retention of insulin as well as by the output of insulin from the β -cells.

In the present investigation the serum insulin and the blood glucose response to glucose ingestion have been simultaneously determined in the portal and in the peripheral venous blood in a group of patients with pancreatitis. The intention has been to evaluate the insulin concentration gradient between the portal and the peripheral venous blood and some of the factors regulating the magnitude of this gradient.

It is generally accepted that pancreatitis may cause a diabetic glucose metabolism. This applies especially to the chronic form (3-24), but even acute pancreatitis may be followed by a reduced glucose tolerance (10, 23-24, 28). Several explanations of these observations have been suggested. Thus diminished insulin secretion by the damaged islet cells (11-13, 14-25, 26), insulin antagonism (17-22) and pro-insulin secretion (22) have been proposed. However normal or even high insulin levels in peripheral venous blood have also been reported (1-17, 22), and in some cases of pancreatitis hypoglycemic symptoms have occurred (9-18, 19-21), occasionally associated with hyperplastic islets of Langerhans (2, 9-19). Since the liver normally extracts a considerable amount of insulin from the blood during a single transhepatic passage (8, 15, 20-27) and additional retention of insulin has also been shown to take place in peripheral tissues (27), the insulin level in peripheral venous blood in response to glucose ingestion is determined by the hepatic and

MATERIAL

The material comprised 12 patients with pancreatitis. One of them (case 11) was woman, 57 years of age. All the rest were men, aged 77-61 years. Three of them (cases 8, 9 and 10) were investigated in connection with their first attack of acute pancreatitis. The remaining subjects had had repeated attacks of pancreatitis (Table I).

Features on which the diagnosis was based were: a) typical symptoms and signs of the disease in all cases, b) increased secretion of amylase in the urine in cases 1, 2, 3, 5, 6, 7, 9, 10, 11 and 12. (at least 512 Wohlgemuth units), c) increased concentration of amylase in the serum in case 8 (6,018 Nörby units), d) pancreatic calcification on X-ray in case 11, and e) histological findings of atrophy in case 4 (Table I).

All the patients had been given normal hospital diet for at least three days before being investigated. In cases 9 and 10, however oral food intake was somewhat restricted for couple of days before the investigations because of nausea. One patient (case 10) was of slight overweight (9% in excess of ideal body weight according to Documents Geigy 1960). Another patient (case 8) had family history of diabetes. None displayed fasting glucosuria. Portal pressure recordings (in our laboratory the portal pressure was below 150 mm H₂O in patients

Table I Laboratory values and diagnostic criteria in 12 patients with pancreatitis

A.P. = acute pancreatitis, S = serum, U = urine, GOT = glutamic-oxaloacetic transaminase, GPT = glutamic-pyruvic transaminase. Normal values: GOT < 40 U/ml, GPT < 35 U/ml

Case no.	Po pressure (mm H ₂ O)	Bilirubin/S (mg/100 ml)	GOT/S (U/ml)	GPT/S (U/ml)	Liver biopsy	Symptoms of A.P.		Increased excretion of amylase/U		Increased conc. of amylase/S on 1 occ.	Pancreatic calcific.	Chronic pancreatitis at autopsy
						On 1 occasion	On > 1 occasions	On 1 occasion	On > 2 occasions			
1	120	0.9	24	25	Normal		x					
2	100	1.3	37	45	Normal							
3	330	0.8	35	50	—							
4	110	0.5	31	36	Normal							
5	130	1.1	22	25	—							
6	140	0.7	70	42	—							
7	90	0.8	16	20	Normal							
8	70	1.2	48	68	Steatosis							
9	80	1.4	92	119	Normal			x				
10	150	1.8	24	50	Normal							
11	160	0.3	15	14	Steatosis							
12	130	0.7	22	31	—							

without hepatic disease (6)) and laboratory data are given in Table I. No portal-systemic shunts could be found at direct portography.

METHODS

Blood glucose was determined enzymatically with a commercial glucose oxidase preparation (Kabi Reagents, Sweden). *Insulino-reactin-azulene (IRA)* was assayed by a double antibody procedure essentially as described by Boedtker and Stone (9). Port insulin (10 μ crystallized) was used for immunization. All insulin concentrations were determined by reference to standard of 2 crystallized human insulin (obtained from Dr J. Schlichtkrull, Novo Research Institute, Copenhagen). The standard deviation for the insulin determinations was, within the same assay 8 and 5.8% of the mean for low and high values, respectively. Liver biopsy specimens were obtained with a Vim-Sil cream needle. Portal catheterization was performed by transumbilical catheterization technique (7, 33) in cases 12. In case 1, transhepatic technique was used (32). In all cases the tip of the catheter was placed in the common portal vein under fluoroscopic control. Glucose was given orally (100 g glucose) after an overnight fast and at least one day after the catheterization procedure. In our laboratory normal peripheral venous insulin values in connection with standardized oral glucose tolerance tests (100 g glucose) are: fasting insulin = 15 ± 10 μ U/ml (mean \pm

S.D.), insulin maximum after 60 min = 89 ± 34 μ U/ml (mean \pm S.D.) (12).

RESULTS

Insulin

Fasting insulin in the portal blood varied between 4–36 μ U/ml (mean \pm S.E.M. = 17.3 ± 3.2 μ U/ml), and in the peripheral venous blood between 2–36 μ U/ml (mean \pm S.E.M. = 9.3 ± 2.7 μ U/ml). The insulin level following glucose ingestion varied considerably interindividually both in the portal and in the peripheral venous blood (Table II). Moreover in some patients the difference between the portal and the peripheral venous insulin levels was substantial, as shown in Fig. 1 and in others very small, as demonstrated in Table II.

Glucose

As shown in Table III the fasting peripheral venous blood glucose concentration did not exceed 100 mg/100 ml except in four subjects (cases 2, 6, 8, 10). All except three (cases 5, 7, 9) however displayed decreased oral glucose tolerance tests (Table III).

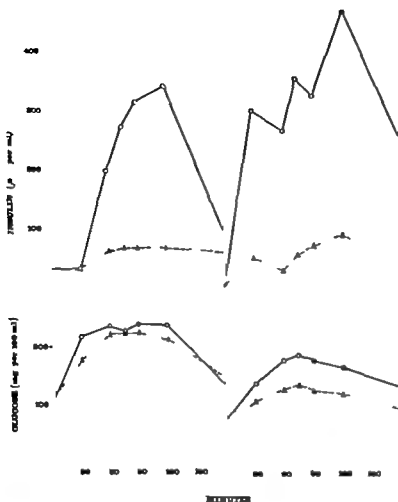


Fig 1 Immunoreactive insulin and glucose in the portal (○—○) and in the peripheral venous blood (△—△) in response to an oral glucose load (100 g glucose) in case 8 (left) and in case 9 (right).

The influence of the portal IRI level on the fractional reduction of insulin between portal and peripheral venous blood (FRI)

In seven individuals (cases 1-4 and 7-9) the IRI in the portal blood reached a level above 99 $\mu\text{U}/\text{ml}$ in response to an oral glucose load (Table II). In each of these patients the sum of all portal IRI values above 99 $\mu\text{U}/\text{ml}$ was formed (ΣI_{po}), and also the sum of the corresponding peripheral venous IRI values (ΣI_{pe}), as shown in Table IV. The individual FRI_x, corresponding to a portal IRI level above 99 $\mu\text{U}/\text{ml}$, could then be calculated with the aid of the formula

$$\text{FRI}_x = \frac{\Sigma I_{pe} - \Sigma I_{po}}{\Sigma I_{po}} \cdot 100$$

In a similar way the individual FRI_x corresponding to a low portal IRI level (<100 $\mu\text{U}/\text{ml}$), was formed. Thus in each of these patients

two separate values for FRI_x were obtained, one corresponding to a high portal IRI level and the other to a low insulin level in the portal blood. As shown in Table IV and Fig. 2, the FRI_x was always higher at the high portal IRI level and proved to be significantly higher when analysis of the paired differences was performed ($p < 0.001$ Student's *t*-test, 2-tailed).

The influence of the glucose concentration on the FRI

In cases 1-10 an attempt to correlate the FRI to the glucose concentration in the portal and in the peripheral veins blood was performed. As shown in Table V no significant correlation existed between FRI and blood glucose concentration. In cases 11 and 12 neither the FRI nor the correlation coefficients were calculated, because in these two cases portal IRI never ex

Table II. IRI in portal and peripheral venous blood before and after oral glucose loading ($\mu\text{U/ml}$)

$$\text{FRI} = (\text{I}_{\text{po}} - \text{I}_{\text{pe}}) / \text{I}_{\text{po}} \cdot 100$$

Case no.		0'	30'	60'	75'	90'	120'	180'
1	Po	8	44	114	80	92	140	186
	Pe	4	54	58	32	24	26	12
	FRI	50	0	49	60	74	81	94
2	Po	18	96	152	344	144	322	88
	Pe	10	34	82	40	36	84	100
	FRI	44	65	46	88	75	74	0
3	Po	18	84	130	158	890	134	—
	Pe	8	42	68	64	70	97	—
	FRI	50	50	48	60	92	28	—
4	Po	24	158	40	132	90	118	118
	Pe	2	48	40	34	38	40	54
	FRI	92	70	0	74	58	66	54
5	Po	10	54	52	—	62	46	42
	Pe	8	22	30	—	30	26	4
	FRI	20	59	42	—	52	44	91
6	Po	26	42	18	28	—	—	—
	Pe	14	20	26	26	—	—	—
	FRI	46	52	0	7	—	—	—
7	Po	36	182	520	—	360	216	52
	Pe	36	118	204	—	162	82	36
	FRI	0	33	61	—	55	62	31
8	Po	36	36	198	272	312	336	92
	Pe	12	36	60	68	68	68	56
	FRI	67	0	70	75	78	80	39
9	Po	14	296	260	344	316	460	248
	Pe	4	46	24	52	68	88	28
	FRI	71	84	91	85	79	81	89
10	Po	4	16	26	34	—	56	42
	Pe	4	20	16	34	—	40	42
	FRI	0	0	39	0	—	29	0
11	Po	8	18	18	22	—	28	—
	Pe	8	12	18	34	—	26	—
	FRI	0	33	0	35	—	7	—
12	Po	8	14	18	22	28	22	22
	Pe	2	12	16	16	18	16	16

ted the range of values seen under fasting conditions (Table II).

DISCUSSION

In chronic pancreatitis acquired β -cell insufficiency has been reported (11, 13, 14, 25, 26). These findings have been based upon a decreased insulin response to β -cell stimulus in the peripheral venous blood.

In the present investigation a low insulin increase in peripheral venous blood following glucose ingestion was not always accompanied by a poor insulin increase in the portal blood but was sometimes due to a substantial reduction of the insulin level between the portal and the peripheral venous blood (Fig. 1 left). Differences in the por-

Table III. Blood glucose in portal and peripheral venous blood before and after oral glucose loading ($\text{mg}/100 \text{ ml}$)

Case no.		0	30	60	75	90	120'	180'
1	Po	68	224	205	195	195	146	117
	Pe	61	298	249	220	176	170	122
2	Po	146	367	413	423	344	344	284
	Pe	116	223	265	265	244	232	197
3	Po	70	154	180	183	189	169	—
	Pe	74	129	146	146	146	146	—
4	Po	102	220	185	171	169	159	159
	Pe	94	185	188	182	172	172	172
5	Po	81	200	216	—	141	122	84
	Pe	90	123	131	—	117	103	84
6	Po	94	190	197	213	—	—	—
	Pe	101	144	188	194	—	—	—
7	Po	75	161	196	—	171	161	81
	Pe	75	134	165	—	118	97	96
8	Po	112	214	231	221	237	233	131
	Pe	116	177	219	219	220	206	146
9	Po	77	135	175	182	173	163	127
	Pe	71	103	124	130	121	117	91
10	Po	101	176	212	212	—	221	201
	Pe	105	130	192	210	—	224	193
11	Po	66	79	146	157	—	201	—
	Pe	81	87	145	145	—	182	—
12	Po	73	162	191	199	223	218	225
	Pe	83	109	162	146	146	150	152

tal blood flow between various individuals might probably explain the interindividual variations in the portal-peripheral venous IRI gradient. Since the liver of man has a considerable capacity for removing insulin from the blood (20, 27), and uptake of insulin by the peripheral tissues has also been reported (27), it seems reasonable to assume that variations in the retention of insulin

Table IV. Influence of the portal insulin level on the fractional reduction of insulin between portal and peripheral venous blood

$$\text{FRI}_L = (\Sigma \text{I}_{\text{po}} - \Sigma \text{I}_{\text{pe}}) / \Sigma \text{I}_{\text{po}} \cdot 100$$

Case no.	Portal IRI level					
	> 100 $\mu\text{U}/\text{ml}$			< 100 $\mu\text{U}/\text{ml}$		
	$\Sigma \text{I}_{\text{po}}$	$\Sigma \text{I}_{\text{pe}}$	FRI_L	$\Sigma \text{I}_{\text{po}}$	$\Sigma \text{I}_{\text{pe}}$	FRI_L
1	440	96	78	224	114	49
2	962	212	78	202	144	29
3	1312	298	77	100	90	50
4	576	176	87	154	80	48
7	1278	566	56	88	72	18
8	1118	264	76	164	104	37
9	1924	306	84	14	4	71

between the portal and the peripheral venous blood might also result in various portal-peripheral venous IRI gradients in different individuals. One patient, with a great portal-peripheral venous insulin difference, also displayed a "diabetic" glucose tolerance curve (Fig. 1 left), while in another patient a considerable insulin gradient between the portal and the peripheral venous blood was found at a time when the glucose tolerance was normal (Fig. 1 right). These findings suggest that factors other than the blood glucose concentration might determine the magnitude of the insulin reduction between portal and peripheral veins. In fact, no correlation was found between

Table V Relationship between the fractional reduction of insulin and the blood glucose concentration

G = blood glucose concentration, r = correlation coefficient

FRI/G ₉₀				FRI/G ₉₀			
Case no.				Case no.			
1	-0.449	7	NS	1	-0.606	7	NS
2	0.567	7	NS	2	0.560	7	NS
3	0.271	6	NS	3	0.196	6	NS
4	-0.423	7	NS	4	0.563	7	NS
5	-0.003	6	NS	5	-0.193	6	NS
6	-0.332	4	NS	6	-0.838	4	NS
7	0.209	6	NS	7	0.377	6	NS
8	0.555	7	NS	8	0.900	7	NS
9	0.401	7	NS	9	0.375	7	NS
10	0.486	6	NS	10	0.484	6	NS

100

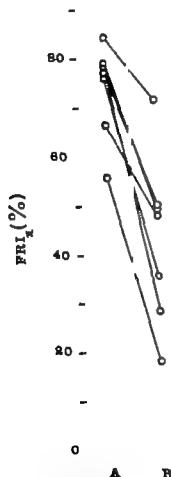


Fig. 2. Individual FRI₁₂ at portal insulin level of >100 $\mu\text{U/ml}$ (A) and <100 $\mu\text{U/ml}$ (B) (cases 1-4 and 7-9).

the fractional reduction of insulin and the glucose concentration in the portal or the peripheral venous blood, as shown in Table V. In this respect our results accord with experimental findings in dogs (15-31).

Samols and Ryder (27) have reported that the proportion of insulin taken up by the human liver seems to remain relatively constant before and during an insulin infusion. It should, however, be noted that liver disease and portal-systemic shunts were present in most of their patients. In our study hepatic vein catheterization was not performed, and as a consequence the portal-hepatic venous IRI difference could not be calculated. Yet the FRI between portal and peripheral venous blood tended to be higher at a high portal insulin level than at a low portal IRI level, as shown in Fig. 2. This observation could be explained by a decline in the portal blood flow following oral glucose. Such a suggestion is, however, unlikely since Gustafson et al. (4, 5) obtained an increase in the splanchnic blood flow following glucose ingestion in normal and gastrectomized subjects. Incomplete mixture of insulin in the portal blood must also be taken into account in this connection. In our present series the tip of the catheter was placed in the common portal vein. Strandell et al. (30) reported adequate portal mixing of ^{125}I in most instances following infusion of the indicator into the splenic artery or superior mesenteric vein. Different concentrations of indicator in different portal branches were, however, seen in one instance (30).

This might mean that a rather small insulin gradient between portal and peripheral venous blood, as shown in cases 10-12 could possibly be due to an uneven distribution of insulin within the portal vein. It could, however also be an indication of seriously damaged β -cells with a small or perhaps abolished capability to increase the insulin production above a basal level in response to an ordinary glucose load. Another explanation of findings as demonstrated in Fig. 2 would be an increased retention of insulin by the liver and/or the peripheral tissues in response to an elevated portal IRI level. Such a proposal is supported by Field et al. (8), who found an increased percentage of hepatic insulin retention when an increased amount of insulin was presented to the liver during intraduodenal or intravenous glucose infusions in dogs. Moreover it has been shown by Katzen and Stetten (16) that an insulin-degrading substance (reduced glutathione) operates in the liver cells. The reduction of glutathione consumes NADPH. This coenzyme arises chiefly from the oxidation of glucose 6-phosphate a process which is insulin-dependent. Thus a scarcity of insulin might result in decreased availability of NADPH and hence diminish insulin destruction. Conversely insulin excess would favour insulin degradation.

In conclusion it has been found in this material that, when the portal IRI level is raised in response to an oral glucose load, an increase in the fractional reduction of insulin between portal and peripheral venous blood appears to be increased. It remains to be resolved, however, whether this observation is valid not only in pancreatitis. Studies in this field are in progress in our laboratory.

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THE INFLUENCE OF PHENFORMIN ON LACTATE METABOLISM IN DIABETIC PATIENTS IN RELATION TO HYPOXIA AND EXERCISE

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Abstract. The influence of phenformin therapy for days on lactate metabolism has been examined in 10 stable diabetics and 1 obese non-diabetic with family history of diabetes in relation to hypoxia and exercise. Phenformin significantly increased the basal lactate and pyruvate production and augmented the production of lactate after hypoxia. During severe exercise and hypoxia phenformin had no additional effect, but the elimination of accumulated lactate, pyruvate and excess lactate was significantly decreased 60-90 min after cessation of the exercise and hypoxia. The effect was most evident in obese and in starved individuals, especially two females. The observed effect of phenformin is regarded as an integrated part of the antidiabetic, hypoglycaemic effect with relation to the decreased ability of the diabetic organism to eliminate accumulated lactate by increasing gluconeogenesis. It is also regarded as potentially toxic effect contributing to hyperlactatemia when other predisposing factors are present.

isotope experiments (16, 17). In the earlier investigations from this hospital the results have been inconclusive (9-23). The purpose of this investigation is to examine the influence of phenformin on lactate metabolism, especially the elimination of lactate accumulated during exercise and by hypoxia. In the present study the patients represented a more homogenous group than previously patients for whom biguanide therapy is commonly used. The results were analysed statistically each patient serving as his own control. Lactate and pyruvate concentrations in the blood were determined and excess lactate concentrations calculated during exercise in an oxygen-poor atmosphere and until 90 min after cessation of the work before and after phenformin therapy of short duration.

The relationship between the mode of action of the antidiabetic biguanides and lactate metabolism has been extensively discussed in the literature for the last 15 years (11-14, 16, 25). The problem is whether the action of biguanides on glucose homeostasis is integrated with an increased lactate production and/or a decreased lactate elimination. Elevated lactate concentrations in the blood have occasionally been observed in diabetic patients treated with phenformin (5, 20, 4, 25) and also in starved normal subjects (19). Furthermore a correlation between the incidence of severe lactic acidosis and phenformin therapy has been suggested (11).

The influence of phenformin on lactate metabolism has been examined in different experimental conditions in humans, including conditions of augmented endogenous lactate production (9, 20, 22, 23-24), different tolerance tests (5, 19) and

MATERIAL AND METHODS

Patients

The investigation comprised 10 stable diabetics, mostly obese individuals and 1 obese non-diabetic with family history of diabetes. The characteristics of the patients are seen in Table 1. The patients were mainly newly discovered diabetics, largely well regulated on diet alone. None of the patients received insulin or sulphonylureas, and they had not been treated with biguanides before. The patients had no apparent cardiovascular or pulmonary disease, nor renal or hepatic insufficiency. Alcohol was not allowed, and the supply of B-vitamins was adequate. No other drugs than phenformin were given. Our patients did not receive ascorbic acid, which was given in the earlier series (23) in order to suppress ketonuria. All the patients were hospitalized and given their usual diet during the experimental period.

Experimental design

The patients are examined in the morning after an overnight fast. Thrombocytopenia was induced by severe

Table 1 Characteristics of the patients

Pat. no.	Sex	Age (y.)	Weight (kg)	Height (cm)	Over weight (%)	Fam. hist. diab.	Previous antidiab. therapy	Remarks
1	♀	39	64	158	12	+		Non-diabetic
2	♀	38	67	157	18	0		Mild retinopathy
3	♂	49	73	168	12	0		
4	♂	55	112	178	56	0		
5	♂	59	77	171	13	0		
6	♂	61	104	182	39	+	Tolbutamide insulin	Mild retino- nephropathy hypertension
7	♂	III	115	180	55	0		
8	♂	50	100	168	34	+		
9	♂	59	74	178	<10	0		
10	♀	41	75	174	<10	+		
11	♂	43	87	190	<10	0		Carcinoma of pancreas ^a

^aDiscovered later at autopsy (the patient was in good condition during the investigation).

exercise in an atmosphere containing 13% oxygen. The exercise was performed on stationary bicycle at a work intensity of 450 kpm/min for the males and 300 kpm/min for the females, a metronome indicating the rhythm. On the day before the first investigation the patients tried the bicycle to get used to it, and we told them all about the procedure. After the investigation on the morning of the first day the patients received phenformin (Dibetol®) in timed-release capsules. The dose was in the upper therapeutic range 100 mg in the evening of the first day 100 mg \times 2 on the second day and 100 mg in the morning of the third day. Then the investigation was repeated under the same circumstances as on the first day.

Lactate and pyruvate concentrations were determined in venous blood collected from an antecubital vein through an indwelling catheter. The basal values were recorded before hypoxia and exercise while the fasting patients were still in bed and while they were sitting on the bicycle. These values were well correlated. Blood was then collected after hypoxia in 13% oxygen for 5 min and during severe exercise in 13% oxygen after 10 and 15 min, whereafter the exercise ceased. Finally determinations were made 15, 30, 60 and 90 min after cessation of the work, when the patients were breathing atmospheric air.

Analytical procedures

Lactate and pyruvate concentrations were determined with a modification of the methods of Hohorst and Bücher et al. (3). The blood was denaturated immediately and thereafter stored at 4°C before analysis on the same day. All determinations were made in duplicate and the concentrations expressed in mmol/L.

Calculations

From the concentrations of lactate and pyruvate the excess lactate concentrations are calculated according to the formula of Huckabee (17). Excess lactate $(L_e = L_a - L_b) = (P - P_b) \cdot L_b/P$ moles/L. L_a and P are the basal concentrations, and L_b and P are the con-

centrations at the time a . The reason for using this parameter is discussed later. For each patient lactate, pyruvate and excess lactate concentrations were recorded at different times before and after phenformin. The differences between corresponding values were calculated and are shown in Tables II, III and IV. A positive difference means more lactate, pyruvate and excess lactate in the blood when phenformin was given than before.

The differences between corresponding lactate, pyruvate and excess lactate values followed the normal distribution and could therefore be analysed statistically on the basis of paired samples by employing the paired t -test. The mean difference and the standard error of the mean (S.E.M.) were calculated at the different times for the total group of patients. From the calculated t -values it could be concluded whether the differences between corresponding values after and before phenformin were significantly different from zero. The null hypothesis was rejected on the 0.05 level.

RESULTS

The results are shown in Tables II, III and IV. The mean basal lactate and pyruvate concentrations corresponded well to those observed by others (10). The mean difference between the basal lactate and pyruvate concentrations was not significant, but the basal lactate/pyruvate ratio showed considerable variation. After hypoxia alone the mean lactate difference was highly significant, whereas the mean pyruvate difference was insignificant. The mean excess lactate difference was of borderline significance. During exercise and hypoxia much lactate, pyruvate and excess lactate was produced whether phenformin had been given or not. In this period and also the first 15–30 min after cessation of the exercise

Table II. Differences between corresponding lactate concentrations after and before phenformin (mmoles/l)

Pat. no.	Basal values	Hypoxia (mm)	Exercise (min)		After exercise (min)				
			5	10	15	15	30	60	90
1	0.14	0.20		0.92		1.33	1.90	1.99	1.91
2	0.21	0.25		-0.39	0.04	1.10	1.11	1.11	1.00
3	0.14	0.10		0.94	1.09	0.98	0.71	0.42	0.30
4	0.19	0.19		0.33	0.84	0.83	0.70	0.62	0.55
5	-0.03	0.00		0.32	-0.17	0.18	0.40	0.27	0.23
6	0.42	0.04		-0.19	-0.15	0.37	0.42		0.23
7	-0.02	0.06		-0.85	-2.09	-1.48	-0.74	-0.13	-0.15
8	-0.01	-0.01		0.28	-0.84	-0.77	-0.45	-0.17	-0.11
9	0.17	0.15		0.19	0.08	0.63	0.51	0.35	0.21
10	0.12	0.16		0.13	0.38	0.26	0.44	0.28	0.25
11	0.16	0.43		0.02	-0.98		-0.03		0.31
No. of									
pts.									
Mean	0.136	0.149		0.155	-0.180	0.343	0.452	0.527	0.450
S.E.M.	0.128	0.128		0.322	0.931	0.874	0.716	0.649	0.579
t	3.905	3.709		0.982	-0.611	1.242	2.092	2.363	2.464
p									
<0.01									
<0.005									
>0.001									
>0.3									
>0.5									
>0.2									
<0.1									
>0.05									
<0.05									
>0.025									
>0.025									

the values showed considerable variation and there were no significant differences. Sixty minutes after cessation of exercise significant results were found for the mean lactate and pyruvate differences, whereas the mean excess lactate difference was of borderline significance. The mean lactate and excess lactate differences were sig-

nificant 90 min after cessation of exercise where as the mean pyruvate difference was of borderline significance at this time.

For some of the patients the values deviated considerably and consistently from the mean differences. This was demonstrated most clearly for the two female patients (nos. 1 and 2) and partly

Table III. Differences between corresponding pyruvate concentrations after and before phenformin (mmoles/l)

Pat. no.	Basal values	Hypoxia (min)	Exercise (min)		After exercise (min)			
			5	10	15	30	60	90
1	-0.006	-0.003	0.021		0.011	0.049	0.069	0.065
2	0.020	0.007	-0.016	0.092	0.049	0.010	0.043	0.030
3	0.023	0.011	0.037	0.028	0.054	0.067	0.031	0.019
4	0.008	0.014	0.016	0.013	0.039	0.052	0.043	0.032
5	0.003	-0.004	0.000	0.024	0.015	0.002	0.011	0.011
6	0.030	-0.015	0.016	0.010	0.002	0.012		0.008
7	-0.002	0.011	0.023	0.004	0.069	0.039	0.000	-0.002
8	0.019	0.004	0.014	0.020	0.040	0.021	0.007	0.024
9	0.002	0.003	0.011	0.003	0.012	0.012	0.030	0.012
10	0.001	0.006	0.021	0.020	0.024	0.014	0.016	0.010
11	0.009	0.027	0.022	0.030		0.010		0.028
No. of								
pts.								
Mean	0.010	0.006	0.006	0.009	0.007	0.017	0.024	0.016
S.E.M.	0.012	0.011	0.020	0.021	0.049	0.033	0.027	0.024
t	0.776	1.470	0.910	1.394	0.575	1.448	2.484	2.142
p								
<0.02								
0.1								
0.3								
0.2								
0.5								
0.1								
<0.05								
<0.1								

Table IV Differences between corresponding excess lactate concentrations (mmoles/l) and basal lactate/pyruvate ratios after and before phenformin

Pat. no.	Basal L/P ratio	Hypoxia (min)	Exercise (min)		After exercise (min)			
			10	15	15	30	60	90
1	3.20	0.03	0.20		0.54	0.66	0.63	0.71
2	0.00	0.21	-0.23	0.06	1.00	0.79	0.66	0.68
3	-2.63	0.15	0.88	1.22	0.65	0.18	0.21	0.30
4	1.09	0.07	-0.03	0.47	0.05	0.02	0.06	0.12
5	-0.93	0.12	0.42	0.24	0.47	0.51	0.48	0.16
6	1.50	0.09	-0.21	-0.76	0.16	0.16		0.22
7	0.02	-0.06	-1.11	-2.03	-0.71	-0.31	-0.14	-0.13
8	-3.54	-0.08	-0.97	0.03	0.25	0.24	0.3	0.49
9	2.76	-0.03	-0.29	-0.32	-0.01	-0.08	-0.18	-0.08
10	1.12	0.02	-0.28	-0.04	-0.13	0.18	0.01	0.07
11	0.84	0.08	0.10	-0.64		-0.02		-0.04
No. of pts.								
Mean	0.312	0.055	-0.138	-0.125	0.27	0.312	0.229	0.237
S.E.M.	2.061	0.089	0.568	0.838	0.475	0.328	0.316	0.291
t	0.502	2.022	-0.807	-0.472	1.511	2.140	2.177	2.591
p	>0.6	<0.1 <0.05	>0.4	>0.6	>0.1	<0.1 >0.05	<0.1 >0.05	<0.05 >0.025

as the amount of lactate above that which would occur if the lactate/pyruvate ratio remained normal. This parameter has been used as an indicator of tissue hypoxia (12, 13), and it is believed that a hyperlactatemia with occurrence of excess lactate could be more dangerous than a hyperlactatemia without excess lactate (11). However the relationship between the excess lactate concentration in the blood and the intracellular redox state is very complicated and not exactly elucidated (1). We did not obtain much further information about lactate metabolism using this parameter instead of lactate alone.

From other investigations one could anticipate an effect of phenformin on lactate metabolism, increasing lactate production (5, 14, 16, 17, 19, 25) and decreasing lactate elimination (5, 18, 19, 22, 23, 25). Our results show that phenformin therapy of short duration increases the basal lactate and pyruvate production and augments the production of lactate after hypoxia. During severe exercise and hypoxia phenformin had no additional effect. The exercise and hypoxia probably are the dominating factors determining lactate production in this experimental situation, phenformin being only of minor importance. Experiments with rats have shown that phenformin at low oxygen tensions contributes less to the hyperlactatemia than hypoxia itself (21). The production of lactate during exercise is, however, difficult to evaluate from this investigation because of the great variation. The decreased lactate production during exercise observed in two cases is difficult to explain. Training has a great influence on muscular metabolism (4, 15), and the possibility must be considered that these patients were better trained when they were examined after phenformin, in spite of the short time interval. Training may modify the action of phenformin on lactate production, which should be remembered in future studies.

Our results showed also that phenformin therapy of short duration had a significant effect on the concentrations measured after the exercise, which—as discussed above—reflects elimination. That phenformin could decrease lactate elimination after severe muscular exercise and hypoxia was shown clearly for the two females and partly for two obese males. Phenformin had a significant effect on lactate elimination 60 and 90 min after cessation of exercise and, in addition, a sig-

nificant effect on pyruvate elimination after 60 min and on excess lactate elimination after 90 min. Exhaustion is probably an important factor predisposing to this effect of phenformin. Furthermore the possibility exists that the diabetic organism is unable to increase gluconeogenesis to the same degree as the non-diabetic organism, because gluconeogenesis is already increased or even maximal in diabetes (7, 8). Lactate is partly eliminated by recycling to glucose (gluconeogenesis) and partly by direct oxidation (1, 8, 17). Inhibition of oxidation—which probably occurs through the action of biguanides (14, 16, 25)—would further decrease lactate elimination in the diabetic organism. A decreased elimination of lactate after phenformin seems very reasonable, especially if other contributing factors are present. In starved non-diabetic normal-weight males phenformin has also an effect on lactate metabolism, which could be interpreted as an inhibition of lactate elimination and/or an increase of lactate production (19).

The observed effect of phenformin on lactate metabolism in this study and other experiments could be regarded as a potentially toxic effect, although phenformin in ordinary therapeutic use is a non-toxic substance (11). The effect could also be regarded as an integrated part of the antidiabetic, hypoglycemic effect (11, 14, 16, 17, 25). An increased lactate production and/or a decreased lactate elimination would tend to reduce the blood glucose concentration. The potential danger of reducing the blood sugar in this way is the risk of hyperlactatemia, possibly with occurrence of excess lactate, and especially when other predisposing factors are present. However it must be emphasized that biguanides also act on other processes in glucose homeostasis not involving lactate metabolism (11). In the reported cases of lactic acidosis associated with phenformin therapy other predisposing factors have often been present (2, 10, 11, 20), but phenformin might have been a contributing factor.

The practical implications of our observations and of the discussion are that phenformin should not be used in patients predisposed to hyperlactatemia and lactic acidosis, for example patients with circulatory failure, hepatic or renal insufficiency and chronic alcoholism. Alcohol intake should always be avoided during phenformin treatment, and severe muscular exercise must not

be performed. Phenformin should not be used in juvenile ketosis-prone diabetics, in whom accumulated lactic acid could contribute to the metabolic acidosis.

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GASTRIC ACID SECRETORY RESPONSE TO PENTAGASTRIN INFUSION IN DIABETES MELLITUS

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Abstract. In order to study the secretory sensitivity which could be a measure of the cholinergic activity in the acid secretory glands, response curves to graded doses of intravenously infused pentagastrin have been recorded for 11 patients with diabetes mellitus and 7 control subjects. ED_{50} is that dose which gives 50% of the maximal response, was calculated. In some patients of the diabetic group the dose response curve is shifted to the right, i.e. an elevated ED_{50} . These findings are interpreted as reflecting reduced cholinergic influences of the stomach.

luminate the secretory sensitivity which could be a measure of the cholinergic background activity in the acid secretory glands (13-20).

MATERIAL

Eleven males with diabetes mellitus, some with ketoacidosis, were selected for the study. None of them had classical manifestations from the gastrointestinal tract. As appears from Table I, their ages ranged from 4 to 57 years and averaged 38 years, while the duration of diabetes varied from 7 to 22 years and averaged 16 years. The majority had less complications of diabetes from the nervous system, and some also from eyes and/or kidneys. All the subjects were in-patients and, at the time of the study, in a chemically satisfactory metabolic state. All were on insulin and diet consisting of 50% carbohydrates, 20% proteins and 30% fat.

The control group comprised eight males of ages ranging from 22 to 44 years and averaging 33 years. No control subject had history of gastrointestinal manifestations or exhibited signs of diabetic peripheral disease.

To avoid gastrosecretory disturbances, neither controls nor diabetic patients received any medication, except as indicated.

CLINICAL METHODS

All patients were examined routinely. The following diabetic manifestations were noted.

1. *Retinopathy* Ophthalmoscopy was performed at the Department of Ophthalmology of the hospital. Retinopathy was graded as follows: 0 = no changes, 1+ = microaneurysms, 2+ = microaneurysms and haemorrhages with or without exudate, 3+ = proliferative retinopathy.

2. *Nephropathy* Coarcted proteinuria (about twice of baseline) (negative bacterial urine culture) was attributed to diabetic nephropathy.

3. *Neuropathy* Routine neurological examination was carried out. At least two neurological signs typical of diabetic neuropathy had to be present for the diagnosis of neuropathy (e.g. Achilles at reflex and impaired vibratory perception in the lower legs).

Rundles (19) reported in 1945 abnormal gastric retention in four diabetic patients. Thirteen years later Kassarjian (11) coined the term gastroparesis diabetorum to denote a condition with similar radiographic appearance which he had found in six patients with diabetes mellitus without clinical signs or symptoms of gastrointestinal distress. In a larger series comprising 35 patients with radiographically large, atonic stomachs, reduced peristalsis and abnormally large retention of contrast medium in the stomach, Zitomer et al. (21) associated these findings with late manifestations of diabetes mellitus and ascribed such disturbances of gastric motility to diabetic neuropathy. The radiographic manifestations have been followed up by studies of gastric emptying, and it has been found that patients with diabetes mellitus of long duration tend to have delayed gastric emptying. Several aetiologies have been proposed, among others degenerative changes in the stomach wall (9).

As any age effects on gastric secretion in diabetes mellitus are very sparsely commented on in the literature, it was deemed interesting to study the relationship between pentagastrin stimulation and gastric acid secretion in order to il-

Table 1 Clinical data on the patients

Case no.	Age (y)	Duration of diabetes (y.)	Retinopathy	Nephropathy	Neuropathy
1	24	9	1+	0	0
2	28	25	3+	+	+
3	30	13	0	0	0
4	31	17	2+	0	+
5	33	20	3+	+	+
6	39	7	0	0	0
7	41	9	0	II	+
8	41	25	1+	0	+
9	50	22	1+	0	+
10	50	13	1+	+	0
11	52	16	1+	0	0

PROCEDURE AND LABORATORY METHODS

The gastric secretory studies were performed in the mornings after 12 hours fasting and abstinence from smoking. A nasogastric tube (Salem Sump Tube) was inserted. The subject, in semi-prone position, was instructed not to swallow. The stomach was drained continuously by a suction pump giving an intermittent pressure of ~50 mmHg. The tube was prevented from blocking by injection of air and intermittent suction with syringe. The gastric juice produced over a 15-min period was pooled. The volume of gastric juice was determined in ml. Its pH was recorded, as was its acid concentration, determined by titration with N/10 NaOH to pH 7.0. The acid output was taken as the product of the gastric juice volume in litres and the acid concentration in mEq/l.

Pentagastrin (ICI) was used as the gastric stimulant. It

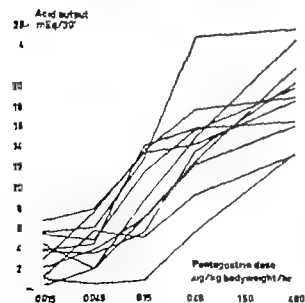


Fig. 1 Response curves to graded doses of pentagastrin for the patients with diabetes mellitus.

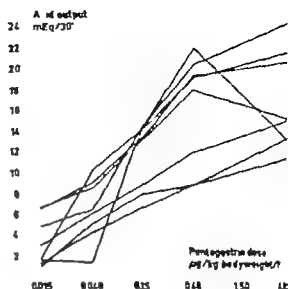


Fig. 2 Response curves to graded doses of pentagastrin for the controls.

was dissolved in N/10 NaCl and infused intravenously with an infusion pump according to the method described by Lawrie et al. (15). The infusions were started at dose of 0.015 µg/kg/h. Three 15-min gastric juice pools were obtained from each subject, but only the two last were used and expressed in mEq/30 min. Subsequently this was repeated four times with pentagastrin doses of 0.048, 0.15, 0.48 and 4.8 µg/kg/h. Dose response curves for pentagastrin were plotted for each subject and used for calculating ED_{50} , i.e. that dose which gives 50% of the maximal response, by linear interpolation.

Wilcoxon test has been used for testing differences between mean values. The differences of the regression lines was tested according to Brownlee (7). A p -value of <0.05 was considered significant.

RESULTS

Response curves to graded doses of pentagastrin for each patient with diabetes mellitus are shown in Fig. 1 and for each control subject in Fig. 2. All the patients with diabetes mellitus exhibited the highest acid output at the highest pentagastrin dose. The same applies to all of the controls but one who had his highest acid response at 0.48 µg/kg b.wt.

Table II reveals that the mean ED_{50} —the effective dose eliciting 50% of the maximal response to pentagastrin stimulation—for the diabetic group significantly exceeded that for the control group ($p < 0.05$). Fig. 3 presents average dose response curves for the two groups. It will be seen that the dose response curves for the group with dia-

betes mellitus are displaced to the right of those for the control group. The secretory responses to the highest pentagastrin doses were higher for the patients with diabetes mellitus than for the controls, while the reverse was the case for the lower pentagastrin doses. The responses for the two groups do not differ statistically in any of the five periods ($p > 0.05$).

The inverted volumes for each 15-min period were plotted against the acidity expressed in regression lines for each group. The difference was not significant ($p > 0.05$).

DISCUSSION

As pointed out previously (14), the method of choice for studying vagal function in clinical practice is the insulin test. Another way of illuminating the vagal component in gastric secretion is to study the sensitivity of the gastric cells to intravenously infused pentagastrin. The significance of cholinergic factors for the acid response of the stomach has been assessed in a number of ways. It is generally accepted that the secretory response to histamine stimulation is reduced after both surgical and medical vagotomy.

Broomé (5) reported that the surgical post-vagotomy reduction could be cancelled by a cholinergic. However Cowley (8) did not find an increase of basal output of acid after vagotomy in the response to carbachol. Broomé et al. (6) expressed the view that the parietal cell response to maximal stimulation is facilitated by a basal release of gastrin and by direct vagal activation of the parietal cells via a basal flow of vagal impulses. Both surgical and medical vagotomy reduce the sensitivity of the parietal cells to submaximal pentagastrin stimulation. The dose response curve shifts to the right and pentagastrin doses that were maximal before vagotomy become submaximal (12).

The pentagastrin dose required for maximal stimulation has been estimated in both healthy subjects and patients with peptic ulcer. There is no agreement on the magnitude of this dose however. Thus Abernethy et al. (2) as well as Aagaard and Schmidt (1) proposed that a dose of at least $6 \mu\text{g/kg/h}$ was required for maximal stimulation. But a small group of 11 volunteers in the above study (2) exhibited a higher response to $5 \mu\text{g/kg/h}$ than to either 2.5 or $10 \mu\text{g/kg/h}$.

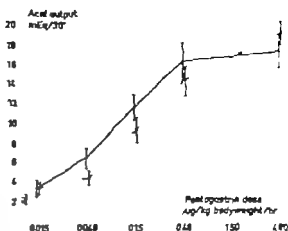


Fig. 3. Response curve to graded doses of pentagastrin for the patients with diabetes mellitus (○) and the controls (—) (mean \pm S.E.).

of pentagastrin Aagaard and Schmidt's highest dose was $6 \mu\text{g/kg/h}$ and the second highest $0.6 \mu\text{g/kg/h}$. Nevertheless Konturek et al. (13) maintained that $1.2 \mu\text{g/kg/h}$ was adequate. Other authors (3, 17) using techniques closely similar to ours, also feel that the latter dose when infused intravenously is sufficient to elicit a maximal secretory response and that in some cases an even lower dose may be sufficient. The highest pentagastrin dose used in the present study is, in our view, that which gives maximal stimulation in healthy subjects. Theoretically however our highest dose might be submaximal for some patients with diabetic vagal neuropathy. If so, a

Table II. ED_{50} —the effective dose eliciting 50% of the maximal response to pentagastrin stimulation

The calculations have been made to three places of decimals

Pat. no.	ED_{50} ($\mu\text{g/kg}$)	Control no.	ED_{50} ($\mu\text{g/kg}$)
1	0.29	12	0.09
2	0.64	13	0.11
3	0.07	14	0.11
4	0.06	15	0.09
5	0.15	16	0.09
6	0.23	17	0.09
7	0.11	18	0.05
8	0.33	19	0.06
9	0.06	Mean	0.09
10	0.21	S.D.	0.02
11	0.26		
Mean	0.22		
S.D.	0.05		

ABSORPTION OF IRON FROM SUSTAINED RELEASE AND RAPIDLY DISINTEGRATING TABLETS

Influence of Daily Numbers of Administrations

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Abstract. The absorption of iron from sustained release preparation given in dose of 100 mg ferrous iron twice daily (at breakfast and at dinner or bedtime) has been compared to the absorption from rapidly disintegrating ferrous sulphate tablet given in dose of 50 mg iron 4 times daily (at breakfast, lunch, dinner and bedtime). The studies were performed in 34 subjects—20 blood donors and 14 patients with iron deficiency anemia. In blood donors the same amount of iron was absorbed from the two types of medication. In patients with iron deficiency anemia significantly more iron was absorbed from the sustained release preparation. There was no indication that the time of intake of the second daily dose of the sustained release preparation had any significant influence on the absorption.

The amount of iron that can be administered in a single oral dose is limited by intolerance symptoms such as nausea and epigastric pain (10). These symptoms are probably related to a high local concentration of ionized iron in the upper gastrointestinal tract. To minimize these symptoms the optimal daily dose of iron with rapidly disintegrating iron tablets is usually divided into 3-4 single doses. By giving the iron as sustained release tablets it seems possible to reduce these types of side-effects and therefore higher single doses may be given. Rybo and Sövell (9) found that 200 mg iron daily of the sustained release preparation used in the present study (Duroferon[®] Durett[®]) gave the same frequency of upper gastrointestinal side-effects as placebo. Thus with this preparation the same daily amount of iron can be administered in a reduced number

of doses. However it is not known whether a reduction in the number of daily administrations may decrease the daily absorption of iron.

Therefore in the present study the absorption of iron from this sustained release tablet given in a dose of 100 mg of ferrous iron twice daily was compared to the absorption from a rapidly disintegrating ferrous sulphate tablet administered in a dose of 50 mg iron four times daily. The absorption studies were performed in 34 subjects comprising blood donors and patients with iron deficiency anemia.

METHODS

A double radioiron method was used to compare the absorption of iron from the sustained release and the rapidly dissolving ferrous sulphate tablets. The sustained release tablets (Duroferon[®] Durett[®]), each containing 100 mg Fe, were labelled with ⁵⁹Fe and the rate of iron release in vitro was about 40% after 1 hour and about 100% within 6 hours. The dissolution tests were made according to beaker method earlier described (4), with 0.1 N HCl as dissolution medium. The ferrous sulphate tablets, each containing 50 mg Fe, were labelled with ⁵⁹Fe and the dissolution of iron in vitro was about 100% within 20 min, determined according to the same method.

The experimental design and details of the analytical procedures were the same as described by Rybo and Hallberg (1). The two kinds of tablets were given on alternate days for 10 days. Two weeks after the last dose blood sample was drawn for determinations of ⁵⁹Fe and ⁵⁵Fe radioactivity in red cells.

The sustained release tablets were given in dose of

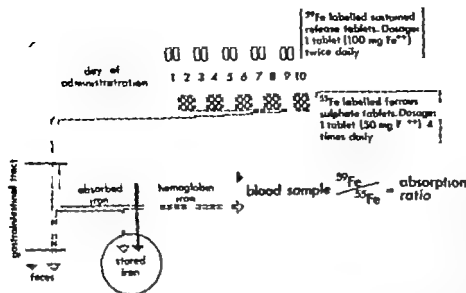


Fig. 1 Experimental design. The two kinds of tablets were given on alternate days for 10 days. Two weeks after the last dose a blood sample was drawn for determination of ^{59}Fe and ^{55}Fe radioactivity in red cells.

1 tablet twice daily—one tablet at breakfast (08.00) and one tablet at dinner or at bedtime (18.00 or 22.00) every second day during 10 days. The corresponding dose schedule for ferrous sulphate tablets was 1 tablet 4 times daily—at breakfast (08.00), at lunch (12.00), at dinner (18.00) and at bedtime (22.00) on the alternate days. Thus the dosage of the two types of tablets was the same 200 mg Fe^{59} daily (Fig. 1).

The statistical calculations were made according to the Wilcoxon rank test and the Mann-Whitney U test.

MATERIAL

Twenty male blood donors and 14 patients (13 females and 1 male) with iron deficiency anemia were included in the study (Tables I and II). Seventeen subjects started the treatment with the sustained release tablets (10 blood donors and 7 patients) and the others with the ferrous sulphate tablets. The blood donors had served as regular donors at least 4–6 times during the last year. They had not received regular iron prophylaxis. In absorption studies using ^{59}Fe -labelled tablets it is important to collect the material during a relatively short period due to the rapid decay of ^{59}Fe . To be able to include in the present study sufficient number of subjects with iron deficiency anemia within short period of time the material was collected from different clinics.

Since the validity of the results from an absorption study of this kind rests upon the patients' adherence to the prescribed therapy precautions were taken to secure an intake of tablets according to instructions. All patients except two with iron deficiency anemia were treated as in-patients and tablet intake was controlled by nurse. Two patients (nos. 28 and 31) and all blood donors were treated outside the hospital. They received both oral and written instructions about the tablet intake and had personal checking box in which every tablet intake and the time for intake were noted. The tablets were individually dispensed in a calendar package on which were printed

day and time for each tablet intake. According to checklist and personal interviews all tablets had been taken. The reported deviations from the time schedule of the tablet intakes were few and there is no reason to believe that they could significantly influence the results.

RESULTS

The results are given in Tables I and II and Fig. 2. The absorption percentage values were calculated from the estimated blood volume (male weight $\text{kg} \times 74$, female weight $\text{kg} \times 65$) (3) and give a correct absorption figure only in case of 100

Absorption ratios

1 mg ^{59}Fe and 1 mg ^{55}Fe tablets, 100 mg ^{59}Fe and 50 mg ^{55}Fe ferrous sulphate tablets 50 mg 4 times daily

on deficiency anemia

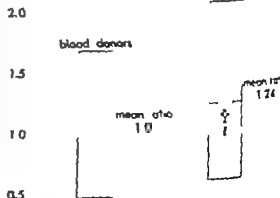


Fig. 2 Individual and mean absorption ratios in blood donors and patients with iron deficiency anemia.

Table I. Absorption of iron in male blood donors from sustained release tablets (100 mg at breakfast and at dinner or bedtime*) and rapidly disintegrating ferrous sulphate tablets (50 mg at breakfast, lunch, dinner and bedtime)

Case no.	Age (y)	Laboratory data before treatment						Absorption			
		No. of blood donations		Hb (g/100 ml)	Hct (%)	Serum iron conc. (μ g/100 ml)	TIBC (μ g/100 ml)	Transferrin sat. (%)	Sust. rel. tabl. 100 mg		Absorption ratio
		Total	Last 12 mo.						2	Ferrous sulph. tabl. 50 mg 4	
1	25	35	7	14.8	43	65	455	14	3.9	3.2	1.22
2	25	11	6	14.8	44	133	408	33	3.2	5.0	0.64
3	24	9	6	14.8	45	59	423	14	4.2	5.4	0.78
4	24	14	6	14.3	43	173	446	37	10.5	8.4	1.25
5	24	13	4	13.3	46	77	445	21	5.5	6.7	0.82
6	III	16	6	14.8	44	79	468	21	4.6	4.2	1.10
7	25	11	5	14.6	43	121	323	37	4.5	5.0	0.90
8	24	22	6	14.3	44	118	364	32	4.5	5.0	0.90
9	24	III	7	14.4	43	63	371	17	7.1	12.2	0.58
10	28	21	5	14.3	43	43	328	13	5.0	4.5	1.11
11	25	14	5	13.4	39	127	432	29	8.1	7.7	1.05
12	25	13	6	15.1	44	73	397	18	4.0	3.7	1.08
13	23	10	6	15.1	46	226	428	53	6.0	7.4	0.81
14	25	16	5	15.8	46	68	261	26	3.5	8.2	0.67
15	25	9	5	16.8	49	214	445	48	3.6	4.2	0.86
16	25	8	5	15.0	44	104	431	24	3.7	3.6	1.03
17	25	7	6	12.6	39	64	415	15	10.5	6.7	1.57
18	25	24	6	15.1	45	63	346	18	7.3	5.5	1.33
19	25	34	6	12.2	40	32	275	12	9.2	7.8	1.18
20	25	41	6	14.9	43	57	357	16	7.9	7.1	1.08
Mean	25	18	6	14.6	44	98	383	25	5.9	6.1	1.00

The second daily dose of the sustained release tablets was given at dinner to subjects 1-10 and at bedtime to subjects 11-20.

utilization of absorbed iron for red cell formation. The absorption ratios given are true expressions of the relative absorbability of iron from the two kinds of tablets.

In blood donors the mean absorption ratio between the sustained release preparation administered in a dosage of 100 mg twice daily and ferrous sulphate tablets administered as 50 mg 4 times daily was 1.00. The mean absorption percentage in the blood donors was about 6% for both preparations.

In patients with iron deficiency anemia the mean absorption figures were 14.0 and 11.7% after the sustained release and the ferrous sulphate tablets respectively. The mean ratio between these two preparations was 1.24. 12 of 14 patients absorbed more iron from the sustained release tablets. The difference in absorbability was statistically significant ($p < 0.01$ according to the Wilcoxon rank test).

The sustained release tablets were given twice daily: at breakfast and at dinner or at bedtime.

These two dosage regimens were chosen as they are commonly used in clinical practice. Neither in blood donors nor in patients with iron deficiency anemia did the times for intake of the second daily dose of the sustained release iron have any significant effect on the absorption.

DISCUSSION

The absorption of iron from the sustained release preparation used in this investigation has earlier been studied by a double radiolabel method in blood donors from the same blood bank (5). It was found that, when comparing a dosage of 100 mg Fe twice daily of the sustained release preparation with the same dosage given as rapidly disintegrating ferrous sulphate tablets, the absorption of iron from the sustained release preparation was significantly increased (ratio 1.29). For both preparations the dosage schedule was one tablet at breakfast and one tablet at dinner.

However optimal iron therapy with rapidly dis-

INHIBITION OF THE NORADRENALINE INDUCED ADENYL CYCLASE STIMULATION BY AUGMENTED α -ADRENERGIC RESPONSE IN SUBCUTANEOUS ADIPOSE TISSUE FROM HYPOTHYROID SUBJECTS

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Abstract. The hypothyroid state in man has previously been shown to be accompanied by decreased lipolytic response to noradrenaline *in vitro*. In earlier publications this diminished response was shown to be due to an enhanced α -adrenergic receptor responsiveness. In order to further elucidate the mode of action of the altered α -adrenergic receptor activity the adenyl cyclase-c-AMP system as studied in fat pads and isolated fat cells from hypothyroid, and in fat pads from euthyroid, subjects. The accumulation of ^3H -c-AMP was measured after labelling of intracellular ATP with ^3H -adenine. Incubations were carried out for 10 min with noradrenaline in the presence or absence of the α -adrenergic blocking agent phentolamine. Noradrenaline caused a significant stimulation of ^3H -c-AMP accumulation in adipose tissue from control subjects, while no significant stimulation was seen in specimens from the hypothyroid subjects. The addition of phentolamine to the noradrenaline-containing media markedly augmented ^3H -c-AMP levels in tissue from both groups. The latter response could not be mimicked by short-term addition of triiodothyronine. The results indicate that the α -receptor controls lipolysis by lowering intracellular levels of cyclic AMP. It is suggested that thyroid hormones might affect the α -receptors in long-term, indirect manner rather than by directly blocking the receptors.

The interrelationship between thyroid hormones and catecholamines has been the subject of a number of studies, the majority of which have been centered around the cardiovascular effects evoked by the two groups of compounds (16). An interrelationship in fat metabolism has also been described. Thus *in vivo* an enhanced lipolysis is observed during catecholamine infusion in patients with thyrotoxicosis, while hypothyroid subjects show a diminished response (4).

In previous publications it was demonstrated that the lipolytic effect of noradrenaline *in vitro* was markedly reduced in subcutaneous adipose tis-

sue from hypothyroid subjects as compared with tissue from control subjects. Normalization was obtained by addition of the α -adrenergic blocking agent phentolamine to the noradrenaline-containing media (14). These results strongly indicated that the α -adrenergic response was enhanced in adipose tissue from hypothyroid subjects. Using glycerol release as an indirect measure of the level of cyclic AMP in the adipocyte it could further be shown that the α -adrenergic receptor response inhibits lipolysis, probably by decreasing the intracellular level of cyclic AMP (1).

The present study was undertaken in order to investigate the effect of the hypothyroid state on the adenyl cyclase-c-AMP system in human adipose tissue.

MATERIAL AND METHODS

Fifteen subjects were studied. Fourteen had developed hypothyroidism after radioactive treatment for thyrotoxicosis and one (no. 5) had had Hashimoto disease. Eight patients were still hypothyroid, while the other seven were on replacement therapy and constituted the control group. Hypothyroidism and effect of treatment are established on the basis of clinical examination and available laboratory investigations. Subject 1 was treated with L-thyroxine 0.25 mg/day while subjects 2, 3, 4 and 6 received 0.15 mg/day. Subject 4 was treated with desiccated thyroid 112 mg/day. Clinical data and the results of the laboratory tests are given in Table 1.

Subcutaneous adipose tissue was excised from the thigh under local anaesthesia using 3-5 ml 0.5% procaine chloride, Clonidine (1). The subjects were non-fasting. The fat tissue specimens were transported to the laboratory in 0.9% NaCl at 37°C.

Lipolysis. The excised adipose tissue was preincubated for 60 min at 37°C in Krebs-Henseleit bicarbonate (KHB) buffer pH 7.4, containing 1% bovine albumin (Fraction B, Armour Pharmaceutical Division). Incubations were

Table 1 Clinical and laboratory findings in the subjects studied

Pat. no.	Age (y)	Sex	TSH (mU/ml)	PBI (μ g/100 ml)	Cholesterol (mg/100 ml)	Reson uptake of T_4 - 125 I (%)	Thyroidal 24-h uptake of 125 I (%)
Control group							
1	52	♀	18	10.0	330	31	—
	59		<1.5	8.7	300	31	—
3	62	♀	12.5	7.3	760	25	—
4	88		13.2	5.6	259	79	—
5*	67		17.3	9.8	11	33	—
6	75		<1.5	8.3	200	31	—
Hypothyroid group							
7	42	♂	400	—	352	24	10
8	44		120	2.2	313	23	4
9	51		195	3.2	304	—	1
10	56		69	2.7	387	21	5
11	59	♀	88	3.3	326	24	20
12	81		103	3.8	479	4	18
13	61	♀	160	7.6	356	23	16
14	66	♀	71	3.4	372	22	7
15	79		100	4.1	339	25	—

Hashimoto disease

then carried out for 6 hours in polyethylene vials (Packard Co. La Grange, Ill.) in KHB buffer pH 7.4, containing 3% bovine albumin and 1 mg/ml glucose at 37°C, using air as gas phase (14). Cholesterol was determined according to the method of Wiskand (17) as modified by Larsen (10).

Adenyl cyclase activity The method described by Kuo and De Renzo (9) was used with some modifications for the assay of adenyl cyclase in adipose tissue sections and in isolated fat cells.

Fat pads Two to three grams of adipose tissue were divided into sections weighing 50–100 mg and preincubated for 45 min with 40 μ Ci of 3 H-adenine (specific activity 40 mCi/mM, New England Nuclear) or 1 mCi of 3 H-adenine (specific activity 611 Ci/mM, New England Nuclear) in 3 ml of KHB buffer, pH 7.4 containing 3% albumin and 1 mg/ml glucose. The pads were washed twice in 0.9% NaCl and once in KHB buffer pH 7.4 containing 3% albumin and 1 mg/ml glucose. The incubations were then carried out at 37°C for 10 min in KHB buffer pH 7.4 containing 3% albumin, 1 mg/ml glucose and 10^{-6} M theophylline, using air as gas phase. The incubation was stopped by placing the vials in an ice-water bath. The tissue specimens were homogenized together with the medium after addition of 0.5 ml of 0.3% perchloric acid and 1 μ moles of cyclic AMP. The homogenate was neutralized and applied on columns containing 0.4–3.3 cm (Dowex 40X-4 (II)) 200–400 mesh. The columns were eluted with water. The fraction containing cyclic AMP was identified by measurement of light absorbance at 260 nm and then further purified with $\text{Ba}(\text{OH})_2$ and ZnSO_4 (8). The supernatant was lyophilized and subjected to paper chromatography as described below.

Isolated fat cells Adipocytes are isolated with collagenase from subcutaneous adipose tissue according to Serru (15). The ATP pool of the cells was labeled with

3 H-adenine during the 60 min of collagenase digestion of the tissue. The cells were washed and incubated in KHB buffer pH 7.4, 4% albumin and 1 mg/ml glucose in freshly subboiled glass tubes. At the end of the incubation 1.5 μ moles of cyclic AMP were added to the tubes and the incubation was stopped by boiling for 3 min. The incubated cells and buffer were extracted with distilled water. The extract was lyophilized and the residue subjected to descending paper chromatography in 3 sec dash systems (room temperature (3): 1) isopropyl-NH-water (7:2:1) followed by 1) isopropyl-NH-water (65:16.7:18.3), and 3) water-saturated *n*-butanol. The recovery of cyclic AMP was determined either by fluorimetry (260 nm) or by the use of 3 H-cyclic AMP. In the latter case the 3 H was added together with the unlabeled nucleotide. Quench correction as made by the addition of internal 3 H-standard when 3 H only was measured, and with external standardization when both 3 H and 3 H were counted.

The following agents were used: noradrenaline bitartrate (Astra, Sweden); phenolamine Replimex (Ciba); triiodothyronine (Sigma); theophylline as obtained commercially.

Clinical test Determination of PBI, cholesterol, the uptake of 125 I-labeled triiodothyronine and thyroid uptake of radioiodine at 24 hours are performed in the usual fasting patients. The values published previously are used for the normal ranges (14). TSH was measured by the double antibody technique described by Oak (11). The normal range in our laboratory being 12.5–40 μ U. Lower values than 1.5 μ U/ml could not be measured.

RESULTS

Noradrenaline at a concentration of 10^{-5} M exerted no lipolytic action in tissue from 8 pa-

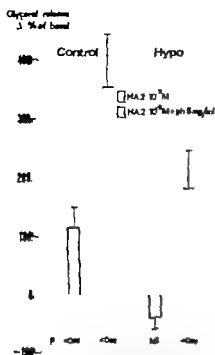


Fig. 1 Lipolytic response (mean \pm S.E.M.) to 2×10^{-9} M noradrenaline (NA) in vitro in the presence or absence of 5 μ g/ml phenolamine (ph) in subcutaneous adipose tissue from five control and five hypothyroid subjects.

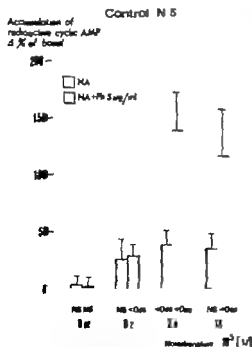
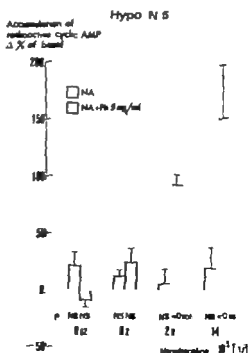


Fig. 2 Accumulation of cyclic AMP (mean \pm S.E.M.) in response to different concentrations of noradrenaline in subcutaneous adipose tissue. The p -values are calculated

thyroid subjects, while a significant stimulation ($p < 0.05$) was obtained in tissue segments from control subjects. Addition of phenolamine (5 μ g/ml) to the noradrenaline-containing media significantly augmented the effect of noradrenaline in tissue specimens from both control ($p < 0.02$) and hypothyroid ($p < 0.05$) subjects (Fig. 1). Phenolamine alone did not significantly influence lipolysis in either the control or hypothyroid group (112.0 ± 23.8 and $103.7 \pm 31.5\%$ mean \pm S.E.M.).

Noradrenaline, in the concentration range of $2-14 \times 10^{-9}$ M, significantly enhanced the formation of cyclic AMP in adipose tissue from control subjects (Fig. 2). In tissue specimens from hypothyroid patients none of the noradrenaline concentrations used stimulated cyclic AMP formation above the basal rate (Fig. 2). Addition of phenolamine to the incubation media markedly increased the accumulation of cyclic AMP in adipose tissue from both groups of subjects, the degree of stimulation in the hypothyroid group now being as high as in the controls (Fig. 2). Phenolamine alone had no such action in either the control or the hypothyroid group (126.1 ± 19.3



as the paired difference between the basal and the stimulated values.

Accumulation of
radioactive cyclic AMP
Δ % of basal

1500-

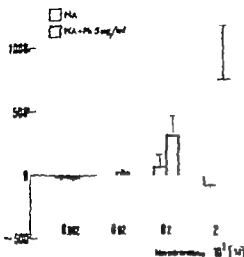


Fig. 3 Effect of different concentrations of noradrenaline (NA) in presence or absence of 5 μ g/ml phentolamine (ph) on isolated fat cells from three hypothyroid subjects (mean \pm S.E.M.).

and $89.0 \pm 20.3\%$ mean \pm S.E.M.) The basal adenyl cyclase activity in the two groups could not be compared since the method used did not allow the determination of absolute amounts of cyclic AMP.

The accumulation of cyclic AMP in isolated fat cells from hypothyroid subjects was not stimulated by noradrenaline (Fig. 3). Again, the addition of phentolamine increased the formation of cyclic AMP (Fig. 3). Addition of triiodothyronine (5×10^{-8} M) to the incubation medium had no significant effect on either noradrenaline or noradrenaline + phentolamine-induced enhancement of cyclic AMP accumulation in adipocytes from hypothyroid subjects (Fig. 4).

DISCUSSION

In a series of publications we have demonstrated that the lipolytic effect of noradrenaline in vitro is obliterated by hypothyroidism in man (12, 13, 14). None of the individual components of the lipolytic system seemed to be deranged in the adipose tissue from such subjects since an adequate lipolytic response could be evoked with isopropyl-noradrenaline, theophylline and dibutyl cyclic AMP. The diminished response to noradrenaline was restored by the α -adrenergic antagonist, phentolamine, and it was therefore concluded that the

defective lipolytic response to noradrenaline was due to enhancement of the α -adrenergic response (14).

In another series of experiments it was shown that α -receptor stimulation decreased theophylline-induced lipolysis, whereas that evoked by dibutyl cyclic AMP remained unchanged (12). These experiments indicated that α -receptor stimulation resulted in a decreased intracellular level of cyclic AMP. However, since the rate of lipolysis gives only an indirect measure of cyclic AMP levels, the above statement cannot be accepted as conclusive.

The results of the present study show that noradrenaline did not stimulate lipolysis in adipose tissue from hypothyroid subjects. However, the addition of phentolamine to the noradrenaline-containing media brought back a lipolytic response to the amine in accordance with our previous studies (12, 13, 14). Furthermore it was shown that noradrenaline in concentrations that effectively stimulate lipolysis in normal adipose tissue did not significantly enhance the formation of cyclic AMP in fat tissue and adipocytes from hypothyroid subjects. Again, inhibition of the α -adrenergic receptors restored the noradrenaline effect on cyclic AMP accumulation. Thus there was agreement between the effect of noradren-

Accumulation of
radioactive cyclic AMP
Δ % of basal

2000-

1500-

1000-

500-



Fig. 4 Effect of triiodothyronine (5×10^{-8} M) on the accumulation of 3 H-cyclic AMP in the presence of 2×10^{-6} M noradrenaline \pm 5 μ g/ml phentolamine in adipocytes from four hypothyroid subjects (I-IV).

alline on lipolysis, on the one hand and that on cyclic AMP formation on the other supporting our hypothesis that the α -adrenergic response is enhanced in the hypothyroid state and that stimulation of this receptor most probably results in lowering of the cyclic AMP level. Since identical results were obtained both in fat pads and in isolated fat cells from hypothyroid subjects, the effect of phentolamine must be located in the fat cells and not in other types of cells, e.g. those of the connective tissue component.

It may be questioned whether α -adrenergic stimulation decreases the intracellular level of cyclic AMP by inhibiting adenylyl cyclase or by stimulating the specific phosphodiesterase(s) which degrades cyclic AMP to 5' AMP. The results presented here were obtained in the presence of theophylline, a potent inhibitor of the phosphodiesterase enzyme (1). However the inclusion of theophylline or other inhibitors does not completely abolish the degradation of cyclic AMP (6). Although our results favor the hypothesis that α -adrenergic stimulation lowers the level of cyclic AMP by inhibition of the adenylyl cyclase enzyme, the possibility of an enhanced breakdown of the nucleotide induced by α -adrenergic stimulation cannot be completely ruled out at present.

The present results are not in agreement with the findings of Krishna et al. (7), which suggested the presence of decreased amounts of adenylyl cyclase in adipose tissue from hypothyroid rats. However the existence of α -adrenergic receptors in rat adipose tissue has been difficult to demonstrate (5), and the effect of hypothyroidism in this species therefore may be different than in man.

Our previous results, as well as those presented here indicate that the difference between adipose tissues from normal and hypothyroid subjects is not a qualitative one, but reflects the enhancement of one of the adrenergic responses. Treatment of the patients with thyroid hormones normalized the adrenergic receptor response (13). Our inability to augment the noradrenaline response by simultaneous addition of T suggests that the effect of replacement therapy is not an immediate one, and does not resemble that of phentolamine.

The concentration of T_3 used in this study seems to be adequate, since the same concentra-

tion has been shown to exert a stimulatory effect on both cyclic AMP formation and lipolysis in rat adipocytes stimulated with adrenaline (2). The absence of a significant effect of T_3 in our experiment might possibly be due to the fact that the cells were not preincubated with the hormone. Such a procedure could have changed the specific activity of the 3H ATP pool and this, in turn, could have invalidated the measurements of adenylyl cyclase.

The present study has shown that α -adrenergic receptor effects in adipose tissue from hypothyroid as well as from normal patients are accompanied by decreased levels of cyclic AMP. In tissue from the hypothyroid group noradrenaline gave no rise in cyclic AMP accumulation or glycerol release, while a stimulation was seen in tissue specimens from the euthyroid group. These data further support the hypothesis of an enhanced α -adrenergic activity in the hypothyroid state, this activity being expressed most probably by direct or indirect inhibition of the cell-membrane-bound adenylyl cyclase enzyme.

ACKNOWLEDGEMENTS

This investigation was supported by grants from Medical Research Committee of the Swedish Life Insurance Companies, the Foundation for Ungradeded Examinations at Karolinska Institute and A. Robert' Foundation.

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DISODIUM CROMOGLYCATE IN BRONCHIAL ASTHMA

A Double-blind Trial in Adult Patients

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Abstract. Twenty patients suffering from pronounced and stable bronchial asthma have been treated with disodium cromoglycate (DSCG) and placebo in a double-blind crossover trial. Significant improvement was obtained in subjective scores as well as in the lung function measured by vital capacity and forced expiratory volume (1 sec) during treatment with DSCG compared to placebo. Toxic effects on the heart, bone marrow, liver or kidneys were not demonstrated. The effect of DSCG is generally modest, but in a few patients the drug had considerable and clinically relevant effect.

Disodium cromoglycate (DSCG) (Fig. 1) was introduced in 1967 as a new drug in the treatment of bronchial asthma (1-15, 17). The drug has since proved to be active also in allergic rhinitis (11, 14, 26) and in exercise-induced asthma (8, 28).

The mode of action of DSCG is not quite clear. *In vivo*, pretreatment with DSCG can prevent allergen-provoked attacks in asthmatics (1, 3, 10, 27). Furthermore DSCG can inhibit the allergic reaction in various experimental models, for instance in passive cutaneous anaphylaxis studies on rat skin with serum from sensitized rats (20, 25), in studies on passive sensitization of monkey skin (5) and of human lung tissue (29) with reagin-containing serum from allergic patients.

In these experiments the inhibition seems to be caused predominantly by a reduced release of histamine from the mast cells, without demonstrable effect on the antigen-antibody interaction *per se* (5, 20, 25, 29). However other mechanisms of action are possible, including a reduced sensitivity to histamine (18).

Metabolic studies mainly on animals have demonstrated that DSCG is absorbed only to a small extent from the digestive tract (7, 22). In animal

experiments the absorption from the lower respiratory tract was almost 100% after 24 hours (23) when inhaled as a powder about 9% of a dose is absorbed in man (7). Injected intravenously the plasma concentration of DSCG is diminished by one half after only a few minutes. The drug does not accumulate in the body but is rapidly excreted through the kidneys and biliary system. Metabolites have not been found (22).

Toxicological studies have not demonstrated any side-effects in asthmatics even during long term treatment with inhalation of DSCG in pharmacological doses (24). Monkeys have tolerated inhalation of DSCG in doses 15-20 times larger than the therapeutic for up to three months without any harm (6). Animal experiments have generally shown DSCG to be relatively non-toxic. However i.v. injection in dogs (10 µg/kg b.wt.) and monkeys (1 µg/kg b.wt.) have caused alterations in blood pressure, heart rate and respiration (5, 6).

Extensive clinical studies on asthmatic patients have demonstrated significant effects of DSCG compared to placebo using a double-blind crossover technique (2, 4, 15, 16, 17, 19, 21, 30). Some authors have reported an effect in 50-70% of the treated patients (4, 16, 17, 21, 30), while others only found DSCG effective in 30-40% (2, 13, 19). Several studies seem to point to an essentially better effect of DSCG in extrinsic than in intrinsic asthma (9, 12, 16, 19).

In the individual patient the effect of DSCG has been difficult to predict, but a few asthmatics have improved essentially subjectively as well as objectively (4, 13, 19, 21).

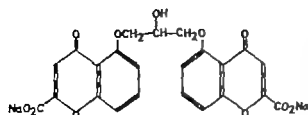


Fig. 1 Dithodum cromoglycate (DSCG).

Because of the discrepancies in the various reports concerning the value of DSCG in the treatment of bronchial asthma, we have found it justified to study the effect of the drug compared to a placebo preparation in a double-blind trial.

PATIENTS AND METHODS

Twenty patients, 14 women and 6 men, aged 19–73 years (average 42.6), suffering from bronchial asthma, were treated with DSCG and placebo in a double-blind crossover trial. All patients had characteristic attacks of expiratory wheezing improved by bronchodilators and were in a stable condition, having at least one attack of asthma a day. Excluded from the trial were asthmatics with negligible symptoms, varying frequency of attacks, exercise-induced or seasonally induced symptoms, severe bronchitis, and furthermore patients who were not capable of recording symptoms reliably on diary cards.

Allergy with positive reaction to relevant inhalation antigens was demonstrated in 10 of 20 asthmatic patients ("extrinsic asthmatics"), while negative reactions were found in 9 patients ("intrinsic asthmatics"). One patient was not investigated for allergy.

Eosinophilia in the blood (more than 400 eosinophils/ μ l)

found in 19 of 20 patients during one or more examinations; several patients showed markedly increased ones.

Treatment with prednisone was given to 16 patients before the trial and continued with unaltered dosage during the entire period. All investigations were carried out on an out-patient basis after an observation period of at least 14 days.

The following parameters were recorded:

1) Lung function tests: vital capacity (VC), forced expiratory volume (FEV₁) and in most cases expiratory peak-flow rate, total lung capacity, functional residual capacity and residual volume. All values were assessed at least twice on each occasion.

2) Blood tests: Hb concentration, ESR, WBC including differential count, thrombocytes, eosinophils, serum bilirubin concentration, prothrombin, glutamate-pyruvate-transaminase, alkaline phosphatase and serum creatinine.

3) Urine: proteins, glucose and blood.

4) ECG

The trial comprised two periods of four weeks each. Inhalation therapy by β -agonists was initiated at random

with either active drug DSCG 20 mg + lactose 20 mg, four doses a day or placebo lactose 35 mg + azkhydron sodium sulphate 5 mg, four doses a day according to the double-blind principle. Coded capsules were supplied from Fibros Limited Pharmaceutical Division, Leicester shire, England. At the end of the first period of four weeks the treatment was crossed over to the opposite drug for the following period of four weeks. In this way 9 patients received DSCG initially followed by placebo, and 11 received placebo, followed by DSCG. The code was not broken until the end of the trial.

Immediately before the trial and after each period of four weeks the above mentioned lung function tests, blood tests, urine sampling and ECG were carried out.

During the trial all patients recorded daily their degree of asthma, dyspnoea and the number of spray inhalations by scores on diary cards. The score for asthma during the day was the number of asthmatic attacks. Scores for dyspnoea in daytime were graded from 1 to 5 according to 1960 questionnaire on respiratory symptoms from Medical Research Council, 1h 1 for no dyspnoea and 5 for loss of breath on least exertion. Scores for use of bronchodilator were the number of times inhaler was used during day or night. Scores for asthma during the night were graded from 1 to 5, 1h 1 for sleep through the night and 5 for kept awake most of the night by asthma.

The patients were controlled clinically at least every fortnight, when the recordings were verified and possible side-effects recorded.

Subsequent to the double-blind trial but after a pause of 14 days, DSCG was resumed in 13 patients who were given 20 mg DSCG four times a day in *per se* treatment. In this way the effect of long-term treatment was evaluated, especially as regards a possible steroid-sparing effect, development of resistance to DSCG and late appearing undesirable effects.

RESULTS

After treatment with DSCG 15 patients, when questioned, stated improvement of symptoms, whereas 5 stated their asthma to be unchanged. After treatment with placebo 5 patients stated improvement, 11 were unchanged and 4 reported increasing asthmatic symptoms. In most cases DSCG had only a slight to moderate effect on the symptoms.

Summing up and comparing the scores on the diary cards for the last two weeks in each 4-week treatment period, improvement of asthma or dyspnoea was recorded by 14 patients during treatment with DSCG and by 2 during treatment with placebo. Four patients recorded unchanged asthma and dyspnoea during the total period of treatment (Figs. 2 and 3 and Table 1).

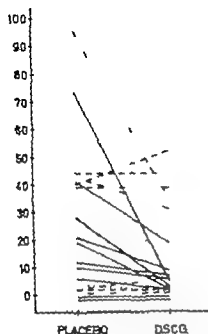


Fig. 2. Sum of scores for asthma during the day for each patient during last 14 days of treatment with placebo or DSCG. — = intrinsic asthma, --- = extrinsic asthma, ... = not classified.

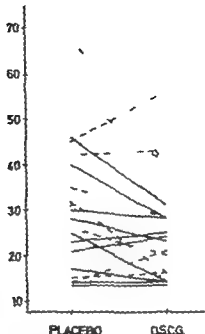


Fig. 3. Sum of scores for asthma during the night for each patient during last 14 weeks of treatment with placebo or DSCG. Symbols as in Fig. 2.

Table 1. Clinical and laboratory data for each patient during treatment with DSCG and placebo

Pat. no.	Type of asthma ^a	Asthma in day-time ^b		Asthma at night ^b		Dyspnoea in day-time ^b		FEV		VC ^c	
		Placebo	DSCG	Placebo	DSCG	Placebo	DSCG	Placebo	DSCG	Placebo	DSCG
1	I	0	0	13	16	18	14	1.73	2.33	4.70	5.22
2	I	11	6	14	21	14	15	2.78	2.98	3.13	3.03
3	E	11	2	46	31	39	16	1.23	2.33	2.92	4.35
4	E	0	0	14	14	28	14	1.30	2.92	3.16	4.23
5	I	37	14	56	40	51	39	1.23	1.40	3.03	3.03
6	E	41	19	28	23	28	23	2.30	2.83	3.30	3.53
7	E	73	5	40	28	28	18	1.18	1.13	2.43	2.43
8	E	12	7	23	25	32	32	1.75	1.95	2.80	2.95
9	I	95	33	60	40	45	38	2.45	2.65	3.45	3.90
10	I	44	44	42	43	44	45	1.10	0.95	1.78	1.80
11	E	0	0	14	14	40	27	1.55	1.48	40	2.60
12	I	0	0	32	16	56	28	1.65	1.58	2.43	3.10
13	E	10	6	21	24	15	17	1.93	1.80	2.30	2.10
14	E	28	3	23	14	34	28	2.03	2.53	2.40	2.15
15	E	6	2	17	14	38	21	Not correct		Not correct	
16	NC	3	1	31	14	26	22	1.58	1.20	3.40	3.35
17	E	21	5	30	28	44	40	1.20	1.45	2.48	2.55
18	I	40	52	43	56	40	46	2.28	2.60	3.35	3.98
19	I	43	31	28	30	41	33	1.70	1.80	2.48	2.45
20	I	59	39	35	28	48	48	0.98	1.33	2.33	2.70

I = intrinsic, E = extrinsic, NC = not classified.

Sums of scores for the last 14 days in each period of treatment.

After 4 weeks treatment.

Table II Patients recording of symptoms during treatment with DSCG and placebo

Parameter	No. of pts.	Scores ^a		DSCG		Wilcoxon ^b Probability ^c
		Placebo				
		Mean	S.D.	Mean	S.D.	
Day asthma	20	25.6	±26.3	13.7	±16.8	$p < 0.01$
Day dyspnoea	20	35.6	±32.5	28.3	±11.4	$p < 0.01$
Day bronchodilator	19	34.2	±32.9	17.3	±19.8	$0.05 > p > 0.02$
Night asthma	20	31.1	±14.8	25.5	±11.7	$p = 0.02$
Night bronchodilator	19	25.6	±27.6	16.4	±17.9	$p = 0.05$

^a Values accumulated during last 2 weeks of each coded period.

^b Matched pairs signed ranks test.

^c Two-tailed.

For all 70 patients a statistically significant improvement of all subjective parameters was shown during treatment with DSCG as compared to placebo (Table II), the symptoms during day-time showing the most significant reduction.

Lung function tests at the end of each period of treatment (Table I and III) also showed significant improvement after treatment with DSCG as regards the FEV₁ and VC ($p < 0.02$) (Table III). The parameters for each patient are shown in Table I and Figs. 4 and 5. Usually the recordings showed only a moderate improvement, but in a few cases a pronounced improvement was seen. The remaining laboratory tests did not indicate any toxic effect on heart, bone marrow liver or kidneys.

Long-term treatment with DSCG in doses of mg four times a day was administered to 13 patients, 10 being on permanent steroid treatment. DSCG made it possible to reduce the dosage

of steroid in 3 patients by 5 to 7 1/2 mg a day but total withdrawal of steroids was not rendered possible. After several months of treatment 2 patients seemed to develop resistance to DSCG possibly because of a complicating infectious bronchitis. However later DSCG seemed to regain its effect in one of these patients.

DISCUSSION

The present study confirms that DSCG is effective, i.e. better than placebo, in the treatment of bronchial asthma in adults. In the group of 80 patients treated with DSCG the subjective symptoms as well as the lung function tests improved significantly regardless of demonstrable allergy in these patients.

Generally the improvement in subjective scores, FEV₁ and VC was only moderate but a few patients showed pronounced improvement in one

Table III Respiratory function tests after treatment with DSCG and placebo

Parameter	No. of pts.	Values ^a		DSCG		Wilcoxon Probability
		Placebo				
		Mean	S.D.	Mean	S.D.	
FEV ₁ ml	19	1.7	±0.5	2.0	±0.7	0.02 > p > 0.01
VC	19	2.8	±0.7	3.1	±0.9	p = 0.02
Peak flow	18	252.7	±104.6	253.2	±113.7	p > 0.05
Total capacity	14	4.8	±0.7	4.9	±0.5	p > 0.05
Functional residual capacity	14	2.8	±0.8	2.7	±0.7	p > 0.05
Residual volume	14	2.3	±0.9	2.1	±0.6	p > 0.05

^a Means of the two consecutive readings taken on each occasion.

^b Matched pairs signed ranks test.

^c Two-tailed.

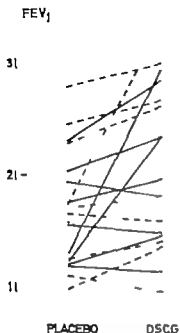


Fig. 4 FEV₁. Means of two consecutive readings for each patient after four weeks' treatment with placebo and after DSCG. Symbols as in Fig. 2.

or more parameters after treatment with DSCG. This observation may be due to a selection of patients with severe asthma, but the same experience has been reported by other investigators (4 13 19 21).

As in previous studies, short-term treatment with DSCG was not followed by demonstrable toxic effects on the heart, bone marrow liver or kidneys.

DSCG thus seems to be a useful supplement in the treatment of bronchial asthma. However clinically the drug is sufficiently effective in only a small number of patients with severe asthma, and the mode of administration—inhalation of the drug as powder—is not commendable.

The effect in long-term treatment and the side effects in man have hardly been sufficiently investigated at present. However DSCG seems to present a new principle in the treatment of some allergic diseases of the immediate type, and for this reason it calls for special interest.

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VITAL CAPACITY

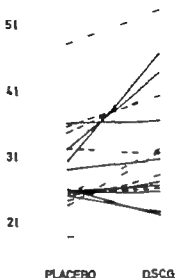


Fig. 5 VC. Means of two consecutive readings for each patient after four weeks' treatment with placebo and after DSCG. Symbols as in Fig. 2.

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RENAL PELVIC CARCINOMA AFTER THOROTRAST PYELOGRAPHY

Case Report

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Abstract. A case of renal pelvic carcinoma, developing in kidney with remaining deposits of thorotrast contrast medium after retrograde pyelography 37 years earlier is described. The patient, 77-year-old man, appeared clinically as an advanced anaemia, caused by chronic pyelonephritis and prostatic hyperplasia, but died within one year with signs of disseminated malignant tumour. An X-ray of the abdomen had shown dense material in the right kidney. Microscopic studies revealed that the contrast was located in carcinomatous tissue in the kidney and autoradiography confirmed that the remaining contrast still emitted α particles.

Thorotrast, a colloidal suspension of $^{232}\text{thorium}$ dioxide, was introduced in clinical practice in 1928 and used for some 20 years as a radiographic contrast medium, both for intravascular administration and for visualization of hollow organs. A number of reports in the literature have considered the long-term hazards of this radioactive substance which, if it is retained in the body will act as a permanent source of radiation. The most frequent complications have been noted from the RES after i.v. administration. In some cases thorotrast has been retained also after intracavitary use, e.g. pyelography (4, 5, 6). The present report describes the clinical and morphological features of a case of thorotrast-induced renal pelvic carcinoma.

CASE REPORT

A 77-year-old man was hospitalized in Oct. 1968 with signs of an advancing anaemia. In 1932 he had been examined because of an attack of urolithiasis. A retrograde pyelography with thorotrast was performed at this time, but did not reveal any concretions. Otherwise he had been in good general health. He was now severely ill, his serum creatinine of 14.5 mg/100 ml. His prostate

was markedly enlarged and there was urinary retention of 600 ml. After relief of the retention and parenteral administration of fluid, electrolytes and blood transfusions gradual fall in serum creatinine down to 4-5 mg/100 ml was achieved. Sixteen days later he had small myocardial infarction. A chest X-ray showed no parenchymal changes. An X-ray of the abdomen revealed diffusely scattered collection of dense material in the right kidney, such seemed to follow the contours of the renal pelvis (Fig. 1). Both kidneys were of normal size and well demarcated. Cystoscopy showed some diverticula and marked trabeculation of the bladder. The prostate was highly enlarged. The urine, as infected with E. coli. The patient condition eventually became better and he could be sent home.

In Aug. 1969 he was again admitted to the hospital because of general deterioration, weight loss and abdominal pains. At the examination there was slight tenderness over the right kidney. The serum creatinine was now 6.2 mg/100 ml. Alkaline phosphatases were moderately elevated. In the right lung rounded homogeneous mass was visible on X-ray. There were no X-ray signs of skeletal metastases, but marrow biopsy showed large areas of malignant tissue, judged as low differentiated squamous cell carcinoma. Faced with unequivocal signs of a disseminated malignant tumour no further investigations were undertaken in the patient in order to establish the primary localization of the neoplastic growth. During the following month new metastatic foci appeared in the lung and the patient died, with respiratory failure and severe skeletal pains.

Autopsy findings. In the right kidney (weight 180 g) the larger part of the renal parenchyma was replaced by neoplastic tissue. The left kidney was small, lobulated and coarsely granulated. The cut surface showed a reduced and diffusely demarcated cortex layer. The papillae are pale and badly delineated. There was moderate hyperplasia of the prostate.

On macroscopic examination the basal change in both kidneys was chronic pyelonephritis with papillary necrosis. The parenchyma was to great extent destroyed and replaced by fibrous tissue, infiltrated by mononuclear cells. Many tubuli were filled with hyaline and

Congress Announcements

Die Europäische Gesellschaft für Gastrokamera-Diagnostik veranstaltet den *V Gastrokamera-Fortbildungskurs* vom 5 bis 7 Oktober 1973 in Eindhoven, Holland, zu dem aus den europäischen Ländern die endoskopisch interessierten Kollegen zur Fortbildung oder zum ersten Kontakt mit endoskopischen Untersuchungen des Oberen Gastro-Intestinums erwartet werden. Die Teilnehmerzahl ist begrenzt. Die Tagung wird in der Technischen Universität in Eindhoven stattfinden.

Auskünfte Dr F Brummer Jac van Maerlant laan 24 Eindhoven, Holland.

International Symposium on the Eye and Systemic Disease will be held in Iowa City Iowa, Nov 15-17 1973

Sponsor The Department of Ophthalmology of the University of Iowa.

Information F A Mausolf M.D., Symposium Chairman, Department of Ophthalmology University Hospitals, Iowa City Iowa, 52242, USA.

The VI International Congress of Infectious and Parasitic Diseases will be held in Warsaw Poland, Sept. 3-7 1974

Official languages. English, French, German, Russian, preferably English.

The programme includes: Zoonoses (epidemiology and course) Hb antigen in acute and chronic

hepatitis, Early diagnosis of infectious diseases, The immunology in parasitic diseases, Chemotherapy of viral diseases, Chemotherapy in bacterial infections, Intensive therapy in infectious diseases.

Deadlines: for an informal application with or without mentioning the title of the report Nov 30, 1973 for abstracts (up to 250-300 English words) Feb. 28 1974

Correspondence IV International Congress of Infectious Diseases, Ul 201 Warsaw Wolska 57 Poland.

The XIV International Congress of Pediatrics will be held in Buenos Aires, Argentina, Oct. 1-9, 1974

Official languages. Spanish, English, French.

Main subjects. The child in its two critical ages I) The newborn II) The adolescent, The child of the present III) The child in the developing world IV) The child in the developed world, The child and the future V) Avenues of progress.

Deadlines: paper with abstract Dec. 31 1973 scientific exhibition Dec. 31 1973 full text manuscript May 30, 1973

Information. Secretario General, XIV Congreso Internacional de Pediatría, Casilla de Correo 3177 Buenos Aires, Argentina.

INTERNATIONAL SOCIETY OF INTERNAL MEDICINE

has elected a new *Executive Committee* during its session in Chicago, on April 12th, 1973. The following were elected officers and members. Lindeboom, Holland, president, Stahl, France; Vannotti, Switzerland, Hughes, Great Britain, Marini, Germany, Blörck, Sweden, Alkan, Israel, and Rosenow USA. Secretary General H. Prel, Switzerland.

THE EUROPEAN ASSOCIATION OF INTERNAL MEDICINE

at its conference in Bonn, on May 5th, 1973 also elected a new *Council*. Its members are. Blörck, Sweden president, Harth, Germany 1st vice president, Dorner France, 2nd vice president, Dagnelie, Belgium, secretary Emerson, Great Britain, treasurer Roos, Holland Sangiorgi, Italy Wegelius, Finland and Schaus, Luxembourg.

EDITORIAL

CRYOGENIC DISEASES

At least in countries with a chilly climate cold is regarded by the lay population as the most important etiologic factor in many diseases—perhaps in competition with trauma. The medical profession has been remarkably critical against such ideas and even patients complaining of obvious cold syndromes, such as rheumatic pain after sitting in a draught, are regarded by many doctors with a somewhat overbearing smile. And still I know of some professors of medicine who had ECGs taken during one very cold winter when they had to keep their left window in the car open and consequently suffered from severe precordial pains for a week or more! They learnt from their own experience the hard way about the reality of rheumatic pain from local chilling. The so-called common cold with coryza is another pertinent example!

One of the reasons, why academic medicine is so reluctant to accept cold as a cause of disease is the fact, that we know remarkably little objectively about the influence of cooling on our tissues and on our humours. Chilblains, trench foot and similar vasomotor disturbances have of course been accepted as cryogenic for many years even if the mechanism is obscure also in these conditions. The fact that icthemia may be a rapid reflex response to cooling is also known from the many persons, who get true angina pectoris on walking against a cold wind.

There are however a number of interesting conditions, where the effects of cooling may be followed and measured on definite substances present in the body. It seems remarkable that these cold sensitive systems are all immunoglobulins but with four different activities. One has been known for many years. It was described by Donath and Landsteiner as a *cold hemolysin*. Most probably this is a monoclonal product of a derepressed line of immunocytes, an essential gammopathy even if very few instances have been properly examined with modern techniques of

protein chemistry. The condition occurs rarely.

The second rare cold syndrome is *cold urticaria*. These patients may develop hives, when they are warmed after cooling. The sequence is the following: cooling, precipitation of an immunoglobulin (γ GK) degranulation of skin mast cells, liberation of histamine urticaria. The phenomenon may be transferred passively by local injection of the cryoglobulin. The exact mechanism of histamine release is not known (1).

The third and so far the best studied is the *cold agglutinin*. It has been found in the plasma as part of a polyclonal response to a challenge with microbial antigens, usually *Mycoplasma*. This antibody is a macroglobulin usually of both kappa and lambda type indicating that several clones of immunocytes are producing the globulin (4). As a matter of fact cold agglutinin disease in the strict sense has all the characteristics of true monoclonal macroglobulinemia but it is interesting that an overwhelming majority of the cases has kappa type immunoglobulin molecules (3). It would be tempting to assume that the kappa configuration of the light chain were a prerequisite for activity as cold agglutinin, but this is obviously not the case as lambda globulins may be quite potent. It is clear that this type of cold agglutinin only reacts with the receptor I on the erythrocyte membrane.

In some cases the titers may become extremely high and the risk for severe hemolytic anemia is evident. Like all "benign" monoclonal gammopathies this is a life-long condition. Treatment with cytostatic drugs does not reduce the titers quickly enough and has never been really effective. A warm climate is the best therapy.

This number of the Acta medica contains an interesting paper on renal disease and *cryoglobulins*. It is appropriate to mention that the word cryoglobulin was coined in 1947 by Lerner and Watson (5) in Minneapolis for a phenomenon

they noted in the serum from a patient with renal disease of at that time unknown origin.

At exactly the same time two Swedish workers, Flemberg and Lehmann (2), noted a myeloma patient, who developed purpura on local cooling and whose serum also showed a precipitate in the cold. This clinical picture was called cold purpura and is now diagnosed as purpura cryoglobulinemica. It is extremely rare.

In 1944 the present author described the first cases of macroglobulinemia in this journal (9) and discussed the fact that one serum gelled on cooling and became increasingly viscous, when cooled from 37 C to 4 C. This high viscosity at low temperatures—and in some case even near normal body temperature—causes a number of symptoms from the circulation and from hemostasis. Gel formation is also regarded as cryoglobulinemia.

This type of cold sensitive immunoglobulin is by far the most common. As a matter of fact it has been shown that small amounts of cryoglobulin may be found in many sera from patients treated in the wards of internal medicine. The reason, why most internists have only seen rare instances, is purely technical. Only if the blood is allowed to flow through a needle directly into a small flask kept in water at 37 C and then transferred to a thermostat at the same temperature will it be possible to find small cryoglobulin precipitates on later cooling of the serum.

The impaired peripheral circulation sometimes—but rarely—causes Raynaud type lesions. The eye grounds is the most characteristic of hyperviscosity and ophthalmological examination should always be carried out in patients with such diagnosis and correct treatment may be life-saving. Cardiac decompensation with this etiology is rare but may be alleviated by plasmapheresis (7). As a matter of fact there is much swelling of the blood and retinal thrombosis is not rare. On the other hand there is also a bleeding tendency chiefly from the mucous membranes in the nose and mouth even when platelet numbers are not decreased, their function is obviously impaired. Plasmapheresis may reverse this picture completely.

It is a fact that many of these cryoglobulins with high viscosity in solution are also cryoglobulins, i.e. they precipitate, when the electrolyte content is lowered, e.g. by dialysis of serum against water

or by dilution with water. This Sia test may be regarded as the least expensive test in clinical chemistry! The same immunoglobulin may have the characters of a cryoglobulin without being a cryoglobulin or a cold agglutinin. The lability of the molecule on cooling or on dilution must therefore have different explanations.

There are many different patterns of cryoglobulinemia and much remains to be investigated with physicochemical, chemical and immunological methods. Such sera may show several different "macroscopical" pictures (8) the simplest is a solid white amorphous sediment on the bottom of the tube. Complete gelification was observed in one of my cases in 1944 (picture of a tube standing upside down without spilling). Usually this gel remains on violent shaking, but in rare instances it is thixotropic and breaks up with the formation of needles. This may have special consequences clinically. The gel may be translucent or opaque. Division into two layers, the upper limpid containing little cryoglobulin, and the lower very viscous with high protein content, has also been observed. The explanation of all these pictures is still lacking. In the study by the Finnish authors the different immunological types of cryoglobulins either consisting of one molecular species, usually macroglobulin or formed by complexes between and IgM acting as a rheumatoid factor (anti-IgG) and IgG molecules, are treated.

The pathology of such immunoglobulin complexes is at present studied very actively by several groups. In some instances such complex formation is connected with maximal hyperviscosity and we have seen one patient with severe symptoms which were relieved by plasmapheresis (6).

Further close observations of such patients will probably give us a lot of valuable information about the problem why cold is unhealthy.

Jan G Waldenström

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SUCROSE FEEDING IN MAN

Effects on Lipolysis and Antilipolytic Action of Insulin in the Adipose Tissue

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Abstract. In four patients with hyperlipoproteinemia and in one with normal serum lipoprotein pattern 800 kcal of the daily caloric intake were replaced by sucrose for 10 weeks. Studies of the adipose tissue metabolism *in vitro* showed that the lipid synthesis from glucose, the basal as well as the noradrenaline-stimulated lipolysis, are considerably increased as compared to the controls. Furthermore, no antilipolytic effect of physiological concentration of insulin was found. It is suggested that sucrose feeding induces changes in adipose tissue metabolism which may be of importance for the increased triglyceride levels noted in these patients.

The mechanisms behind the endogenous (carbohydrate-induced) hyperlipidemia are basically unknown. It is well known, however that in patients with endogenous hyperlipidemia the degree of hyperlipoproteinemia can be modified by the diet. Furthermore, an increase in the plasma triglycerides is a normal response in almost every subject on a high carbohydrate diet.

In a recent study of patients with or without hyperlipoproteinemia (3) 800 kcal of the daily caloric intake were replaced by sucrose for 14 days. It was reported in that study that the sucrose feeding increased the triglyceride level, the plasma insulin level as well as the adipose tissue lipoprotein lipase activity in the normolipoproteinemic subjects. The latter effects would tend to inhibit lipid mobilization from the adipose tissue as well as to increase the elimination of the triglycerides. Since the increase in the hepatic glyceride-fatty acid synthesis was not of a sufficient order of magnitude to explain the increased triglyceride level it seems that the effect of sucrose

feeding on adipose tissue metabolism should be studied. In the present communication some initial results of such studies are presented.

MATERIAL AND METHODS

The five patients subjected to sucrose feeding were admitted to the hospital for an operation of uncomplicated gall bladder disease. The liver function was normal according to our previous definition (3). None of the patients had any known disease apart from the gall bladder disease and in pertinent cases hyperlipoproteinemia. Clinical data of the patients are given in Table I. The plasma lipoprotein pattern was classified according to Fredrickson and Lees (7) using lipoprotein electrophoresis on agarose gel (13).

The patients subjected to sucrose feeding were hospitalized for 20 days. In the morning after the day of admission fasting capillary blood and heparinized venous blood samples were drawn for subsequent determinations of plasma insulin (10), triglycerides (4) and for lipoprotein electrophoresis (13). The patients were then given sucrose, 200 g/day from the third day through day 17. The ordinary hospital diet consists of an average of 500 kcal, distributed as 500 kcal proteins, 900 kcal fat and 1100 kcal carbohydrates, mainly as starch. With the aid of dietician approximately 800 kcal (protein 160, fat 290 and carbohydrates 350 kcal) of the daily food intake were replaced by 200 g sucrose. Throughout the sucrose feeding period blood samples were drawn for the determination of triglycerides and plasma insulin. The patients were operated upon on day 19 after overnight fasting. Anesthesia was given as hexobarbital, nitrous oxide, oxygen and succinyl choline. Specimens of subcutaneous adipose tissue were obtained as soon as the abdominal cavity had been opened. The results obtained with these specimens are in the present study compared to the results previously obtained in this laboratory with specimens from patients who had not been subjected to carbohydrate feeding. Clinical data of part of the control material has

Table I. Clinical data of patients subjected to sucrose feeding for 14 days

Subject no.	Before sucrose feeding		After sucrose feeding
	Lipoprotein pattern	Triglycerides (mg/100 ml)	Triglycerides (mg/100 ml)
1	N	123	112
	II A	131	190
3	II B	232	355
4	IV	198	233
5	II B	344	344
Mean		206	267

Classified according to Fredrickson and Lees (7).

already been described in detail (11). These patients were operated upon for uncomplicated gall bladder disease or exploratory laparotomy after overnight fasting.

The incubation procedure *in vitro* has been described in detail previously (15). Briefly small fragments, total weight 300–500 mg, were incubated in 2.0 ml Parker's medium 199 (Statens Bakteriologiska Laboratorium, Stockholm, Sweden) modified to a glucose concentration of 1.0 mM for 2 hours at pH 7.4 in the presence of 0.15 μ C D-(1-¹⁴C) glucose (New England Nuclear Corp. Frankfurt am Main, West Germany) and with or without the indicated concentrations of noradrenaline (Astra, Söderlilje, Sweden) or insulin (crystallized pork insulin, Vitrum, Stockholm, Sweden). The release of glycerol was taken as an index of the lipolysis and was determined enzymatically (12). The lipids were extracted with chloroform:methanol (2:1 v/v) as described by Folch et al. (6) and the radioactivity in the chloroform phase was determined in Packard Tri-Carb liquid scintillation spectrometer. Quenching was corrected for by means of

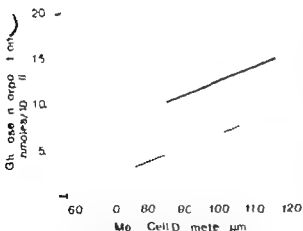


Fig 1. Incorporation of (1-¹⁴C) glucose into the total lipids of specimens of adipose tissue obtained in connection with sucrose feeding (— regression line $y=0.16x-3.46$) and in the controls (--- regression line $y=0.16x-9.40$). Mean cell size was determined on isolated cells.

60.0—

25.0—

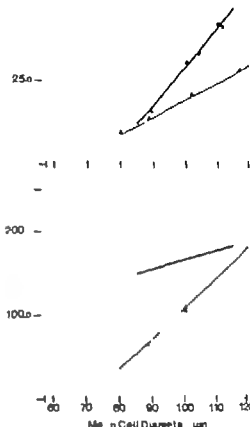
Glyc ol (mean nmole $\times 10^3$ cells)

Fig 2. Top. Basal lipolysis in specimens of human adipose tissue obtained in connection with sucrose feeding (— regression line $y=1.14x-84.11$) and in the controls (--- regression line $y=0.51x-32.12$). Bottom. Increment in glycerol release induced by 5×10^{-6} M noradrenaline in specimens from sucrose-fed individuals (— regression line $y=1.06x+61.62$) and in the controls (--- regression line $y=3.61-251.84$). Mean cell size was determined on isolated cells.

internal standardization. Glyceride glycerol as determined according to Carlson (4). Mean cell size as determined on cells isolated with collagenase as described in detail elsewhere (16).

RESULTS

Since it now seems well established that adipocyte size is an important parameter for the rates of lipid synthesis and lipid mobilization in human adipocytes (1, 5, 8, 11) the data from the controls as well as those obtained after sucrose feeding were related to the mean cell size of the specimens. The control material consisted of individuals who had not been subjected to the carbohydrate-rich diet. Clinical data of part of this material has previously been published (11).

Table II *Effect of insulin on the noradrenaline-stimulated lipolysis in specimens of human adipose tissue obtained after sucrose feeding for 14 days*

Subject no.	Glycerol release (nmoles/10 ⁶ cells)	
	Noradrenaline	Noradrenaline and insulin
1	212.1 ± 9.2	219.0 ± 34.1
2	240.3 ± 11.6	244.1 ± 6.1
3	149.1 ± 10.0	185.1 ± 5.8
4	224.3 ± 8.2	225.1 ± 1.3
5	140.7 ± 2.3	134.8 ± 1.3

The incubations were performed for 2 hours in the presence of noradrenaline ($5 \cdot 10^{-6}$ M) and insulin (10^6 μ U/ml), respectively. Results \pm S.E.M. of duplicate determinations.

Lipid synthesis from glucose. In agreement with previous studies from carbohydrate-fed individuals (14) the rate of glucose conversion to lipids was for all cell sizes considerably enhanced as compared to the controls (Fig. 1). Addition of insulin (10^6 μ U/ml) increased the incorporation of glucose by about 25% in the specimens with the smallest mean cell size.

Lipid mobilization. Sucrose feeding seems to increase the basal lipolysis considerably and particularly in the larger adipose cells (Fig. 2, top). Furthermore, the glycerol increment induced by noradrenaline was considerably greater in the specimens obtained after sucrose feeding (Fig. 2, bottom). The apparent convergence of the regression lines is due to one subject (no. 5). Thus the rate of glyceride turnover in adipose tissue increased after sucrose feeding.

The antilipolytic effect of insulin at physiological concentrations (10^6 μ U/ml) was also studied. As shown in Table II no effect of insulin at all was noted in the specimens from the sucrose-fed individuals. In the control material this concentration of insulin consistently inhibited the noradrenaline-stimulated lipolysis by at least 25–30% (11).

DISCUSSION

In a previous report (3) it was found that the sucrose feeding for 14 days used in the present study resulted in increased levels of plasma insulin and triglycerides. It was also reported that the lipoprotein lipase activity rose, which would lead to an increased uptake of triglycerides in the

peripheral tissues. This finding, together with the fact that the increase in the fatty acid synthesis in the liver was rather limited, makes the adipose tissue a probable site for the provision of the substrate for the lipoprotein synthesis.

Most previous studies of human adipose tissue in connection with dietary manipulations have been concerned with the effect on lipid accumulation rather than lipid mobilization. Although no study with rat adipose tissue in connection with carbohydrate feeding is directly comparable to the present study it seems that the results obtained have varied between different laboratories. Braum and Fábrý (2) reported that a high carbohydrate diet increased the lipolysis and the adenyl cyclase activity while Gorman et al. (9) were unable to find this. Our findings of an increased lipolytic response would be consistent with an increased adenyl cyclase activity in connection with sucrose feeding. An unexpected finding was the complete loss of antilipolytic action of insulin at a concentration at which insulin has been consistently found to exert this action (11). The reason for this "insulin resistance" in connection with sucrose feeding is unknown. Since a stimulatory effect was found on the site of lipid accumulation, this dissociation of the actions of insulin does not seem to be due to any change in the insulin-receptor interaction.

To summarize, it seems that sucrose feeding leads to an increased rate of basal as well as noradrenaline-stimulated lipid mobilization, a loss of antilipolytic effect of insulin, while the effect of insulin on the side of lipid accumulation is maintained. From these results it would seem that sucrose feeding induced changes in adipose tissue metabolism which may be of importance for the increased triglyceride levels noted in these patients. However since four of the five patients studied had hyperlipoproteinemia, it is not possible at present to draw valid conclusions whether the changes reported are specific for patients with a derangement in their lipoprotein pattern. The recent studies of Tashiro and Matsuda (17) on the metabolism of adipose tissue from rats with hyperlipidemia due to experimental nephrosis seem to be of relevance for this question. It was shown by these authors (17) that fat cells from nephrotic rats had increased rates of lipid mobilization and, in addition, insulin failed to exert an antilipolytic action. It may well be, then,

that changes in adipose tissue metabolism are of importance for the development of the hypertriglyceridemia noted in different conditions. Further studies designed to elucidate these questions are at present in progress.

ACKNOWLEDGEMENTS

This investigation was supported by the Swedish Medical Research Council (project B73-03X 3506) and the Swedish Life Insurance Companies.

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MYDRIASIS OF UNUSUAL CAUSE IN A THROMBOCYTOPENIC, ANEMIC WOMAN WITH TEMPORARY AMAUROSIS

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Abstract. A 30-year-old female developed after curettage for menometrorrhagias high intracranial pressure, choked optic discs with ocular bleedings, amaurosis and mydriasis. The cause is obscure and the explanation of the mydriasis is unusual and to our knowledge not previously described.

Papillary edema without proven intracranial lesion has previously been reported in different hematologic disorders, such as iron deficiency anemia (7-10), pernicious anemia (13), polycythemia vera (9) and thrombocytopenic purpura (17). In some of these cases cerebral symptoms have been pronounced. Watkins et al. (17) described three such cases with thrombocytopenia and anemia. These patients were all young women. The thrombocytopenia was diagnosed owing to severe menometrorrhagias. Besides the papillary edema of 2-5 dioptres there were retinal hemorrhages. These authors concluded that the anemia secondary to the thrombocytopenia was the real cause. The increased intracranial pressure was a reaction to cerebral anoxia.

A similar case has recently been seen by us. Since the patient had a complication previously not described, the explanation of which is unusual, we feel it worthwhile to report.

CASE REPORT

A 30-year-old woman was admitted to nearby hospital because of heavy menstrual bleedings. Menses had been irregular and extensive since menarche. During pregnancy at the age of 18 there was albuminuria. In the last 6 months prior to partus. At the age of 23 endometriotic cysts were removed. Salpingitis as diagnosed half a year later.

Present illness and hospital course

Because of severe menorrhagia uterine curettage was performed on Nov. 14, 1969. At the time the Hb was

8.0 g/100 ml. In connection with the curettage 500 ml blood transfusions (450 ml each) were administered. The patient also received trimethoprim and (C)liclopiroxil and ampicillin (Ampipenil). A mild noncharacteristic headache appeared couple of days before the curettage. The pain was accompanied severely during the day following the procedure. During 10-day period the patient developed almost complete amaurosis and pronounced hemorrhages in the retina as well as in the vitreous body. The cerebrospinal fluid (CSF) was clear and contained only few white and red cells of fresh appearance probably due to the puncture. The protein content was normal (40 mg/100 ml). The opening pressure was 300 mm H₂O with the patient in the horizontal position.

An ophthalmoscopic examination revealed complete mydriasis with minimal reaction to light. Papillary protrusion was observed and found to be 5 dioptres on the right and 6 dioptres on the left. Peristaltic laboratory studies on Nov. 21 showed the following values: Hb concentration 12.9 g/100 ml, WBC 7100/mm³, 10% normal differential cell count, platelets 10000/mm³, bleeding time 8 min and coagulation time 3 min. On the 15th day the patient was transferred to the University Hospital of Uppsala.

The remarkable findings on physical examination were then maximal mydriasis without any reaction to light and almost complete amaurosis. There were no petechial bleedings on the body except for few spots in the upper gum. No stiffness of the neck was found. The pupils reflexes were normal. No adrenergic or hepatogastric reflexes were noted. The patient was obese weight 77.1 kg and height 168 cm.

The ophthalmologist found large and widespread hemorrhages located both in the pre and peripapillary regions (Fig. 1). The papillary protrusion was estimated to be 5-6 dioptres bilaterally. The course of the recovery of vision is shown in Fig. 2. The diagnostic work-up of the patient gave the following results.

Radiological studies

X-rays of the chest, abdomen and skull were negative. A pneumoencephalography was performed. There were no signs of intracranial expansion. Brain scanning using Technetium 99 M was also normal.

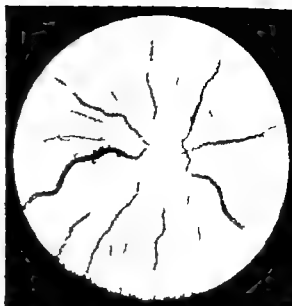


Fig 1 Eye fundi on Dec. 3 1969 (left) and on Aug. 28, 1970 (right).

Laboratory data

Hb concentration was 10–13 g/100 ml during the course. RBC was normal. WBC was moderately increased with slight shift to the left in the differential count. Reticulocytes are elevated with maximum of 7.1%. Platelets changed as seen in Fig. 2. ESR was 48–1 mm/h. The smears of bone marrow aspirate showed an approximately normal number of megakaryocytes. They had a well defined granularity of the cytoplasm, but no platelet formation, as seen in Werthof's disease. Liver function tests were like normal limits. Direct and indirect Coombs' tests were negative. Haptoglobin was 116 mg/100 ml. Several tests for ANF were negative. The serum electrophoresis showed slight decrease of albumen and a slight increase of γ -globulin.

The results of the endocrinological studies, including

plasma proteins-bound iodine level, triiodothyronine test, catecholamines and 5-hydroxyindole acetic acid, are all within normal limits.

Treatment and course

Dexamethasone (Decadron) in high dosage (24 mg a day during the first week, gradual reduction of the dosage during the second week) was given in an attempt to decrease the intracranial pressure. Later this drug was replaced by prednisolone in the hope of obtaining an increase in the number of platelets. The treatment was, however, only temporarily beneficial as regards the thrombocytopenia and after 10 weeks the spleen was removed. The spleen weighed 188 g. The microscopic picture showed follicular hyperplasia with all marked regeneration centres in the malpighian bodies. The dose-

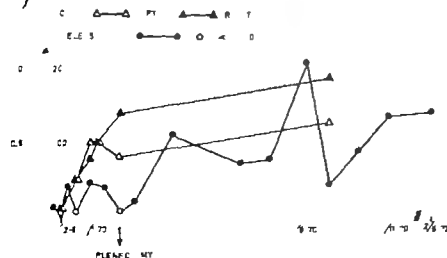


Fig 2. Visual acuity and platelet counts during the course.

oids were dilated. The findings were compatible with the diagnosis of Wertheof's disease. The microscopic examination as performed by G. Hultqvist. In the meantime the hemorrhages in the eyes cleared, the pupillary protrusion decreased gradually and the vision improved considerably (Fig. 2). After the splenectomy recovery was uneventful. Initially platelets increased. After few weeks, however, the platelets again decreased and amikloprine (Imurel[®]) as given together with prednisolone. The patient is later discharged with gradually reduced dosage of prednisolone and 140 amikloprine 300 mg/day.

In Aug 1970 the patient had follow-up study. The visual acuity was almost normalized as seen in Fig. 2, but the patient spontaneously complained of blurred vision for few moments when she looked from close to faraway object or *vice versa*. A Micholy[®] test was performed. The result will be discussed in detail later.

The physical examination was normal. Platelet counts 40 000 and 45 000/ mm^3 on two occasions. The other laboratory work-up was essentially normal. The patient was discharged with prednisolone 5 mg and amikloprine 100 mg/day. Platelet counts in Sept. and Nov 1970 88 000 and 134 000/ mm^3 respectively.

At the last follow up (May 1972) the patient was in good general condition. Hb concentration 15.5 g/100 ml and platelet count 143 000/ mm^3 . Amikloprine as well as prednisolone has been discontinued since half year. Her menstrual bleedings have not returned, except once in the summer of 1971.

DISCUSSION

Thrombocytopenia and retinal hemorrhages in combination with signs of elevated CSF pressure have previously been reported (17).

The case presented here had this combination together with complete pupillary paralysis, which has not earlier been reported to our knowledge. A possible explanation of the mydrasis will be discussed. The following discussion will deal with the papilledema, the visual loss and the pupillary paralysis.

The papilledema

The marked pupillary protrusion, the finding of an elevated CSF pressure, the demonstration of a normal entricular system on pneumoencephalography enable us to use the label "benign intracranial hypertension" or "pseudotumor cerebri" in this case. A number of abnormal conditions have been noted in association with the clinical syndrome pseudotumor cerebri, e.g. endocrine disturbances, anemia, chronic respiratory insufficiency heart disease, allergic conditions, non-cerebral infections, etc. A thorough review of the literature on suggested etiological factors has been

given by Hagberg and Sillanpää (6). To avoid unnecessary negations we confine ourselves to stating that, after a careful and systematic interrogation of the patient and after a number of laboratory tests, there are only three factors of possible etiological significance in the present case which are worth discussing: the anemia, the long history of menstrual dysfunction and the obesity of the patient. Pseudotumor cerebri has been described in association with anemia caused by iron deficiency (7-10), vitamin B₁₂ deficiency (13) hemolysis (6) or blood loss (15).

The pathogenetic mechanism in pseudotumor cerebri associated with anemia is usually thought to be hypoxia damaging the blood-brain barrier and the membrane function of the brain cells with cerebral edema as a result (6). Although it cannot be excluded that bleeding into the optic nerves had occurred in the present case, thus adding to the papilledema, it seems very unlikely that the increase of CSF pressure was caused by diffuse or localized bleeding into the brain—the patient was mentally completely normal, she had no lowered level of consciousness, there was no focal neurological deficit, EEG examination revealed only generalized changes such as slow α -activity and generalized symmetrical slow background activity she had a normal brain scan.

Although we wish to stress the blood loss anemia in the etiological discussion, the history of menstrual dysfunction and the obesity of the patient could be of importance. Both menstrual dysfunction (4) and obesity (1, 3, 5, 8, 12) have been reported to be in some way connected with the syndrome of pseudotumor cerebri. Endocrinological studies have suggested the possibility of a defect in the pituitary-corticotropin adrenal axis in benign intracranial hypertension (11). An endocrine dysfunction could act by creating a cerebral edema (6). In our patient a possible endocrine disturbance could thus have been at least a predisposing factor for the development of a cerebral edema during her anemic period. Another suggested etiology of benign intracranial hypertension is antibiotic therapy. It has been claimed, for instance, that tetracycline (?) and penicillin (14) may cause the condition.

The visual loss

Papilledema, for instance in cases of intracranial tumor is usually not accompanied by loss of vi-

sion. In our case the impairment of vision is probably explained by the extensive intraocular bleedings.

The pupillary paralysis

The most puzzling feature in the clinical picture of our patient was the maximal mydriasis and the loss of pupillary reflexes. No pupillary dilating agent was ever administered. Both the reaction to light and the reaction to nearsight were lost in both eyes. On the 22nd day a minimal reaction to light was first seen. There was still no reaction on convergence. The visual acuity was at this time 0.1–0.3 bilaterally.

Two weeks later the pupils were still mydriatic. There was a slightly improved reaction to light but no reaction on convergence. The visual acuity was now 0.4 in the right eye 0.5 in the left. After another week the pupils were normally wide, they reacted normally to light and nearsight. The visual acuity was 0.5 bilaterally. The pupils had been extremely dilated with loss of reaction on convergence for five weeks. The reaction to light had been lost for more than three weeks.

Pharmacological causes of the pupillary paralysis can be excluded. *Amauric pupillary paralysis* does not explain the fact that the reaction on convergence was lost. Furthermore it is not a good explanation of the complete loss of light reaction and the extreme mydriasis when the visual acuity was 0.2–0.3. *Bilateral damage to the third nerve* is unlikely because there were no signs of paralysis of the extraocular muscles. A *midline lesion* situated in the midline in the periaqueductal gray matter near the rostral end of the aqueduct could theoretically cause bilateral complete paralysis and dilatation of the pupils. As our patient had a thrombocytopenia, one could speculate about a small bleeding in this area, causing the pupillary signs. In practice, however vascular disease affecting this area almost always produces associated signs such as nuclear ophthalmoplegia, paralysis of upward gaze, loss of convergence, exotropia, and other defects of ocular movement (16).

We think that the final clue in the search for the localization of the responsible lesion was given by the patient at a follow-up examination in Aug. 1970. She complained of blurred vision for a few moments every time she looked from a near object to a faraway object or vice versa.

This was a new symptom, she had never experienced it before her illness. She had thus developed *tonic accommodation*—a symptom occurring in conditions which are caused by damage to the postganglionic parasympathetic fibres destined for the iris and ciliary body (16).

Further evidence of the postganglionic localization was given by the Mecholyl® test. This test is commonly used to confirm the diagnosis "tonic pupil" (pupilotonia). It is based on the principle that a partially denervated end organ becomes supersensitive to the transmitter substance. Thus the partially denervated iris sphincter in Adie's syndrome reacts with intense miosis to conjunctival administration of a 2.5% solution of Mecholyl® while Mecholyl® in less than a 15% concentration has no noticeable effect on the normal pupil (16).

Although instillation of Mecholyl® (2.5%) failed to induce miosis in our patient, she complained that her far vision was blurred for some hours afterwards, near vision was normal. Unfortunately the refraction was not determined after instillation of Mecholyl®. The reaction on Mecholyl® demonstrated a denervation supersensitivity of the ciliary body i.e. postganglionic lesion.

The short ciliary nerves carry among other fibres, postganglionic parasympathetic fibres to the iris sphincter and ciliary muscle. They pierce the sclera at the posterior pole of the globe near the entry of the optic nerve and run anteriorly first in the sclera and then in a plexus in the subchoroidal space to their ultimate destinations in the anterior structures of the eye (16).

We assume that in our patient the short ciliary nerves were damaged by edema and/or hemorrhage at the point of their entry into the globe or possibly more distally in the wall of the eye, and that this nerve damage was the cause of the pupillary paralysis. It could of course be argued that the Mecholyl® test did not demonstrate a denervation supersensitivity of the iris sphincter but only of the ciliary muscle, i.e. the result of the test contradicts our assumption that the pupillary paralysis was due to a postganglionic nerve lesion. The evidence of damage to the short ciliary nerves still persists. Obviously a complete reinnervation of the iris sphincter had taken place at the time when the Mecholyl® test was performed, while the ciliary muscle was still partially denervated.

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Table I. Clinical data

Fasting blood glucose concentration (mg/100 ml)	No.	Sex (♂) (♀)	Age ^a (yr)	% of average b.wt. ^{a,b}
Diabetics				
<90	11	8 3	64±2.7	95±2.8
90-129	12	6 6	67±2.5	101±3.7
130-199	11	7 4	63±2.8	94±3.4
>200	16	5 5	67±1.8	95±4.5
Non-diabetics	12	9 3	65±1.5	95±2.7

^a Mean ± S.E.M.^b Documenta Geigy Scientific Tables, 6th ed., p. 623

METHODS

The diabetics were arbitrarily subdivided into 4 groups according to their fasting blood glucose concentrations (mean of fasting blood glucose concentrations before the four tolerance tests): fasting blood glucose <90 mg/100 ml, 90-129 mg/100 ml, 130-199 mg/100 ml, >200 mg/100 ml. The corresponding 2.5-hour blood glucose values were: 143 ± 6.1 , 203 ± 13.1 , 396 ± 22.0 and 473 ± 22.8 mg/100 ml.

Patients with fasting blood glucose below 90 mg/100 ml had no diabetic symptoms and no treatment was given. In the group with fasting blood glucose between 90 and 129 mg/100 ml one third had symptoms and were subsequently treated with carbohydrate fixed diet. In the group with fasting blood glucose between 130 and 199 mg/100 ml half of the patients had symptoms and were treated with carbohydrate fixed diet and oral antidiabetic drugs. All the patients with fasting blood glucose higher than 200 mg/100 ml had symptoms and were treated with carbohydrate fixed diet and oral antidiabetic drugs or insulin.

Insulin response pattern of the diabetics was compared with that of 12 non-diabetics of nearly identical age and percentage of average body weight (Table I).

The definition of normal glucose tolerance in the present study was: fasting blood glucose concentration below 100 mg/100 ml and blood glucose falling to below 120 mg/100 ml within 2.5 hours after 100 g of orally administered glucose. Patients whose blood glucose concentration had not fallen to below 120 mg/100 ml within 2.5 hours were called diabetics. None of the patients had signs or symptoms of primary adrenal or pituitary pancreatic diseases.

The following schedule was used:

Day 1, 2 and 3 All the subjects were on a standardized diet containing 300 g carbohydrate and 2000 calories per day.

Day 4 I. glucose tolerance test. 100 g glucose (50% solution) was injected into an antecubital vein in the course of 4 min. After 2 min of injection stop-watch was started and blood was drawn every 10 min for 1 hour.

Day 5 Single oral glucose tolerance test. 100 g glucose

(33% solution) flavoured with lemon were ingested in 3-4 min after drawing the fasting sample.

Day 6. Double oral glucose tolerance test. 100 g glucose (33% solution) flavoured with lemon were ingested in 3-4 min after drawing the fasting sample and the load was given 1 hour later.

During the oral tests blood was collected every 15 min in the first hour and every 30 min in the last two hours.

Day 7 Intermittent.

Day 8 I. tolbutamide test. 1 g (5% solution) sodium tolbutamide was injected in the course of 2 min. After 1 min of injection a stop-watch was started and blood was drawn every 10 min for 1 hour.

All tests were done in the ward but the patients were up and about and not bed-resting except during the night. The tests were performed in the morning after 12 to 14 hours fast (water was given ad lib.) and (ii) the subjects in recumbency.

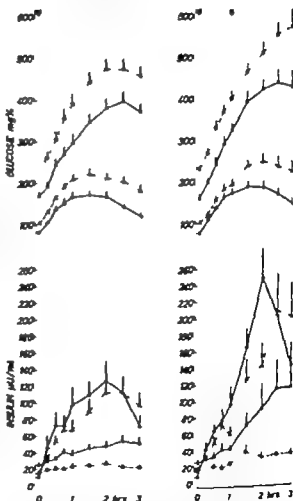


Fig. 1 Blood glucose and plasma insulin curves (mean \pm 1 S.E.M.) after single (left) and a double (right) oral glucose load in 4 groups of diabetics—fasting blood glucose <90 mg/100 ml (\circ — \circ), 90-129 mg/100 ml (Δ — Δ), 130-199 mg/100 ml (\square — \square), >200 mg/100 ml (\diamond — \diamond)—and in group of non-diabetics (shaded area) (mean \pm S.E.M.).

Antecubital venous blood was obtained from an indwelling plastic catheter (length 15 cm). Whole blood glucose was determined by the glucose oxidase method (5) in single determinations. Plasma insulin was determined in triplicate by slight modification of the radioimmunoassay of Hales and Randle (9) after addition of EDTA. The blood samples were centrifuged and stored at -20°C until analysis.

Statistical calculations were carried out with the Spearman rank correlation (12), Page's significance test for linear ranks (13) and the Wilcoxon test (19). The 5% level was accepted as indication of significance.

RESULTS

Fig. 1 shows the plasma insulin response to a single and to a double oral glucose load in non-diabetic subjects. The normal plasma insulin response to oral glucose is characterized by a rapid initial plasma insulin response with the maximal value between the first and second hour after 100 g of glucose. The plasma insulin curve declines from the maximum towards the fasting level within the third hour after the glucose load. After the double glucose load the maximal insulin value is higher but occurs at the same point of time. The plasma insulin curve parallels the corresponding glucose tolerance curve.

Fig. 1 also shows the plasma insulin responses of the 4 groups of diabetics. It is evident that the plasma insulin responses consist of a spectrum of insulin responses. Patients with the mildest glucose intolerance have a normally rapid initial insulin response and a higher than normal insulin level during the last hour of the oral tests. The 2.5-hour plasma insulin concentrations of the pa-

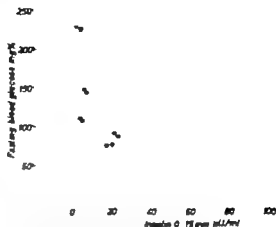


Fig. 2. Correlation between fasting blood glucose concentration and mean increment in the two oral tests from fasting to 15-min plasma insulin concentration in all the diabetics ($p = 0.001$, $R = -0.716$).

tients with fasting blood glucose concentrations < 90 mg/100 ml are significantly higher than the 2.5-hour plasma insulin concentrations of the non-diabetics ($p < 0.05$) during both oral tests. In the patients with blood glucose concentrations from 90 to 129 mg/100 ml this was only the case during the single oral glucose tolerance test ($p < 0.05$). In patients with more pronounced glucose intolerance the plasma insulin response is proportionally delayed initially and the maximum and late values are lower. Patients with the severest glucose intolerance exhibit a very sluggish initial insulin response and a very low maximum value. Even in the patients with the severest glucose intolerance

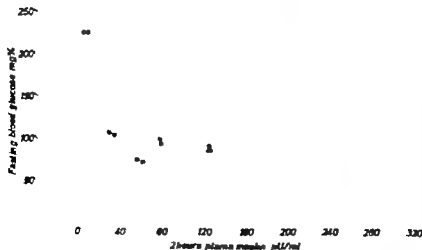


Fig. 3. Correlation between fasting blood glucose concentration and the increment from fasting to the 2-hour plasma insulin concentration during the single oral glucose load in all the diabetics ($p = 0.001$, $R = -0.7576$).

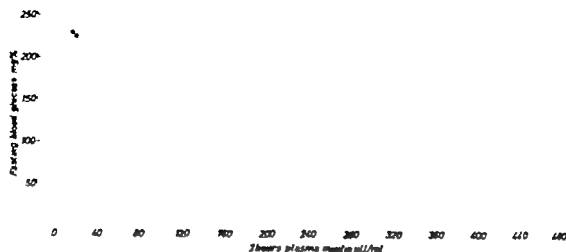


Fig. 4 Correlation between fasting blood glucose concentration and increment from fasting to the 2-hour plasma insulin concentration during the double oral glucose load in all the diabetics. ($p=0.001$ $R=-0.7601$)

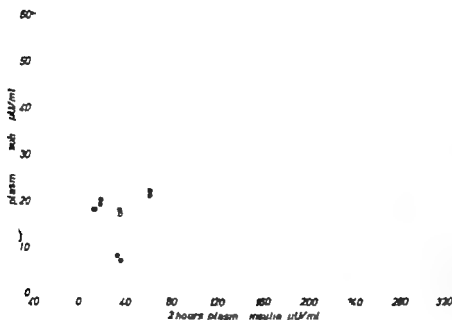


Fig. 5 Fasting and increment from fasting to the 2-hour plasma insulin plot from the single oral test, showing no correlation ($p=0.65$).

ance, however the rises in plasma insulin are significant ($p<0.001$). A negative correlation was found between the fasting glucose concentration (mean of all four tests) and the initial plasma insulin rise in the diabetics (mean increment in the two oral tests from fasting to 15-min plasma insulin concentration) as shown in Fig. 2 ($p=0.001$ $R=-0.7216$).

Figs. 3 and 4 show the inverse correlation be-

tween the fasting blood glucose concentrations (mean of all four tests) and the increment from fasting to the 2-hour plasma insulin concentration in the diabetics during the single ($p=0.001$ $R=-0.7576$) and the double oral glucose load ($p=0.001$ $R=-0.7601$). It is obvious that the negative correlations only exist at fasting blood glucose concentrations higher than approximately 100 mg/100 ml.

There is no correlation between the fasting and the increment from fasting to the 2-hour plasma insulin concentration in the diabetics during either of the oral tests ($0.1 > p > 0.05$) (Fig. 5).

Fig. 6 shows the plasma insulin response to i.v. glucose and tolbutamide in non-diabetics. The plasma insulin response in non-diabetics is characterized by a rapid initial plasma insulin release with the peak value 10 min after the i.v. injection, and thereafter the plasma insulin response declines towards the fasting level—faster after tolbutamide than after glucose.

Fig. 6 also demonstrates the plasma insulin response in the diabetics to i.v. glucose and tolbutamide. Largely the same pattern as during the oral test is seen. Patients with the mildest glucose intolerance show the fastest and highest initial plasma insulin response, and vice versa. During the i.v. glucose tolerance test there is a significant inverse correlation ($p = 0.001$ $R = -0.7713$) in the diabetics between the fasting blood glucose and the increment in the plasma insulin concentrations from the fasting to the 10-min values (Fig. 7) and the case is the same in the tolbutamide test (Fig. 8) ($p = 0.05$ $R = -0.3623$). Again this correlation is restricted to fasting blood glucose values higher than approximately 100 mg/100 ml.

Fig. 9 shows that there is a negative correlation in the diabetics between the fasting insulin concentration and the increment from fasting level to the 10-min insulin concentration in the i.v. glucose

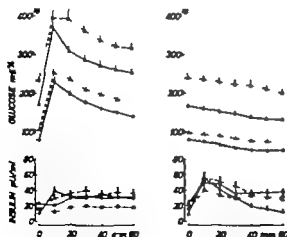


Fig. 6. Blood glucose and plasma insulin curves (mean \pm S.E.M.) after an i.v. glucose (left) and i.v. tolbutamide test (right) (symbols as in Fig. 1) and in a group of non-diabetics (shaded area) (mean \pm S.E.M.).

tolerance test ($p = 0.001$ $R = -0.5150$). This is, however, not the case in the i.v. tolbutamide test ($p > 0.1$) (Fig. 10).

Fig. 11 shows that there exists a positive correlation ($p = 0.01$ $R = +0.5312$) in the diabetics between the fasting blood glucose and fasting plasma insulin concentrations (mean of all four tests). The group of patients with fasting blood glucose concentration higher than 200 mg/100 ml, however, fits badly in the correlation.

There is no correlation in the rather limited number of non-diabetics between the fasting blood glucose and the fasting plasma insulin concentrations (mean of all four tests), fasting blood glucose and the increment from fasting to 15-min and 2-hour plasma insulin concentrations in the oral tests or from fasting to 10-min plasma insulin concentration in the i.v. glucose tolerance test. There is, however, a significant positive correlation between the fasting blood glucose and the increment from fasting to 10-min plasma insulin concentration in the i.v. tolbutamide test ($p = 0.02$, $R = +0.6907$).

DISCUSSION

This study has demonstrated that the plasma insulin responses of non-obese, middle-aged and old diabetics constitute a spectrum of responses varying with the metabolic state. More than a decade ago Yalow and Berson (20) showed that the plasma insulin response to oral glucose in mild maturity onset diabetes is characterized by an initial delay but the average insulin concentration



Fig. 7. Correlation between fasting blood glucose concentration and increment from fasting to 10-min plasma insulin concentration in i.v. glucose tolerance test in all the diabetics ($p = 0.001$, $R = -0.7713$).



Fig. 8 Correlation between fasting blood glucose concentration and increment from fasting to 10-min plasma insulin concentration in i. tolbutamide test in all the diabetics ($p=0.05$ $R=-0.3623$).

at 2 hours exceeded the highest average concentration in non-diabetics. Since then the delay in the initial plasma insulin response has been the most emphasized common abnormality seen in all types and degrees of diabetes mellitus and even in pre-diabetics (4 15 18). It has recently been documented that this concept does not include at least the most common type of diabetes mellitus, i.e. patients with glucose tolerance test diabetes (normal fasting blood glucose concentration and a diabetic glucose tolerance test). These patients, who are found in great number in every diabetes detection survey have a normal initial plasma insulin output after both oral and i.v. glucose and

after i.v. tolbutamide (10 17). The present study has shown that the insulin response pattern after oral glucose seen in the mildest degree of glucose intolerance is a sustained rise in plasma insulin concentration and a delayed decline towards the fasting level with a higher than normal plasma insulin level during the last hour of the oral test. The initial plasma insulin release is, however quite normal. Thus the group of diabetics with the mildest glucose intolerance shows a tendency to normal appearance of the plasma insulin curve but with a delayed decline from the maximum towards the fasting level. Patients with more pronounced glucose intolerance have a delayed initial insulin output, and those with the most severe glucose intolerance have a very weak and slow plasma insulin rise.

After i.v. glucose and tolbutamide largely the same pattern is seen as during the oral tests. With increasing carbohydrate intolerance the initial plasma insulin output is seen to be more and more sluggish and the peak disappears, while only small alterations occur during the later part of the insulin response.

A significant positive correlation is found between the fasting blood glucose and the fasting plasma insulin concentrations in the diabetics. Patients with the severest intolerance, however fit badly in the correlation. This seems to suggest that elevated blood glucose in the fasting state elevates the fasting plasma insulin concentration

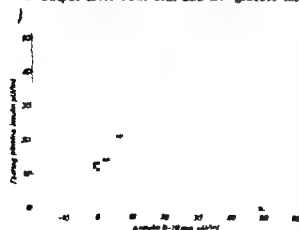


Fig. 9 Correlation between fasting plasma insulin concentration and increment from fasting to 10-min plasma insulin concentration in i. glucose tolerance test in all the diabetics ($p=0.001$ $R=-0.5150$).

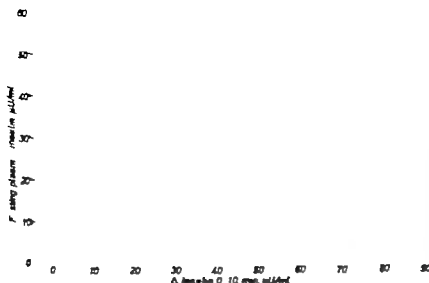


Fig. 10 Fasting plasma insulin concentration and increment from fasting to 10-min plasma insulin concentration in 1. self-administered test in all the diabetics, showing no correlation.

In the severest cases the β -cells are exhausted and the fasting plasma insulin level falls again. Bagdade et al. (1) was not able to demonstrate such a correlation between the fasting insulin and fasting blood glucose concentrations.

In contradistinction to Bagdade et al. we do not find a positive correlation between the fasting plasma insulin and the plasma insulin response in the diabetics in any of the oral tests. On

the contrary there is a tendency to an inverse correlation ($0.1 > p > 0.05$). By recalculating the figures of Bagdade et al. it is found that this correlation is only present when obese and non-obese diabetic subjects are lumped together and does not exist in the non-obese diabetic subjects ($p > 0.1$). In addition we find a significant inverse correlation between the fasting and the 10-min plasma insulin concentrations in the i.v. glucose tolerance test in the diabetics.

In the diabetics a significant positive correlation obtains between the fasting plasma insulin and the fasting blood glucose concentrations. A significant negative correlation is found in the diabetics between the fasting blood glucose level and the plasma insulin response expressed as the increment from fasting to the 15-min and the 2-hour plasma insulin concentrations in the oral test and from fasting to the 10-min plasma insulin concentration in the i.v. tests.

To conclude, our findings show that patients with the mildest degree of diabetes have no defect in the initial insulin release mechanism, but the sustained and higher than normal later rise points to some kind of insulin resistance operating in these patients either in the blood or at the tissue level. In patients with more pronounced glucose intolerance the initial plasma insulin response is sluggish, and in the severest case of glucose intolerance an overall diminishing of the insulin response is found.

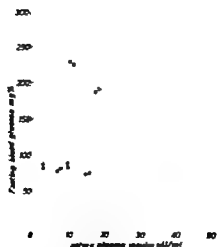


Fig. 11 Correlation between fasting blood glucose concentration and fasting plasma insulin concentration in all the diabetics ($p = 0.02$, $R = +0.3613$) and in diabetics with fasting blood glucose < 200 mg/100 ml ($p = 0.01$, $R = +0.5312$). O = fasting blood glucose > 200 mg/100 ml.

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EFFECTS AND SIDE EFFECTS OF TREATMENT OF HYPERCHOLESTEROLEMIA WITH CHOLESTYRAMINE AND NEOMYCIN

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Abstract Thirteen patients with primary type II hyperlipoproteinemia have been treated with neomycin and cholestyramine, alone and in combination with clofibrate, during 6-week periods in a cross-over study lasting 36 weeks. Neomycin, 2 g daily and cholestyramine, 24 g daily produced significant median 23% and 27% reductions in plasma cholesterol respectively. Cholesterol was further but non-significantly reduced by addition of clofibrate 2 g daily. Dyspeptic side-effects were severe, necessitating withdrawal of 7 and 4 patients from treatment with neomycin and cholestyramine respectively.

Reduction of plasma cholesterol in patients with primary type II hyperlipoproteinemia (2), or essential hypercholesterolemia, can be accomplished by a wide variety of drugs (7). Cholestyramine is an anion exchange resin which, administered orally, increases the fecal excretion of bile acids (4 & 9). Presumably because of an increased conversion of cholesterol to bile acids, and in spite of increased cholesterol synthesis (3 & 9) a reduction of plasma cholesterol is usually obtained. It is one of the safest and one of the more reliable cholesterol lowering drugs, but side effects often render continued treatment difficult. Neomycin has been reported to lower cholesterol substantially with few side-effects (10). In conjunction with clofibrate it has been found particularly useful (11). Its mechanism of action is subject to controversy and may be unrelated to its antibiotic properties (12, 13).

The purpose of the study was to compare the effects and side-effects of cholestyramine and neomycin, alone and in combination with clofibrate, in the treatment of primary type II hyperlipoproteinemia.

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MATERIAL AND METHODS

The material comprised 13 patients with isolated elevation of plasma cholesterol, with normal triglyceride levels, and with type II patterns on paper electrophoresis of plasma lipoproteins (5). In 10 cases the disorder was shown to be familial by finding first degree (9 cases) or second degree (1 case) relatives similarly affected. Patient 8 had insulin-dependent diabetes mellitus, and patient 9 had previously had slightly increased alkaline aminotransferase and alkaline phosphatase interpreted as an expression of alcoholic liver damage. On the basis of the personal and family history these additional disorders were considered to be without relation to the hypercholesterolemia. No other metabolic disturbances were found in screening procedures including measurements in plasma or serum of glucose, uric acid, thyroxine, alkaline phosphatase, alkaline aminotransferase, albumin, and immunoglobulin A, G and M, as well as testing of urine for glucose and protein. All patients had normal serum creatinine. Age and sex of the patients are seen in Table I, which also illustrates the design of the study.

The trial period for each patient was 36 weeks, subdivided evenly by 6. The patients were randomly allocated to 2 groups. After a control period of 6 weeks, patients 1-7 were started on neomycin and patients 8-13 on cholestyramine. After 6 weeks clofibrate was added. Following a second control period, cross-over was performed. Neomycin was given as neomycin sulfate 2 g daily divided into 2 or 4 doses according to individual preference. Cholestyramine was given as Questran® 24 g daily divided into 3 doses. In the clofibrate periods, 2 g of this drug (Atrofen®) in 2 or 4 daily doses were given. The patients were encouraged to continue treatment in spite of possible side-effects, and they were instructed to adhere to their customary diets. They are treated on an out-patient basis.

At 2-week intervals blood samples are drawn in the morning after an overnight fast for the determination of cholesterol, triglycerides, phospholipids, hemoglobin, leucocyte counts, alkaline aminotransferase, alkaline phosphatase and creatinine. Cholesterol was measured in heparinized plasma by the method of Abell et al. (1). Serum triglycerides were determined by Laurell's method (6) at the Department of Clinical Chemistry. Urine was tested for glucose and protein, and body weight was controlled

Table I. Results of treatment with neomycin and cholestyramine separately and in combination with clofibrate

Mean plasma cholesterol (mg/100 ml) in consecutive 6-week periods and percentage reduction of plasma cholesterol (within parentheses) in relation to preceding period

Pat. no.	Age (y)	Sex	Control 1	Neomycin	Neomycin + clofibrate	Control 2	Cholestyramine	Cholestyramine + clofibrate
1	53	♀	490	448 (8.6)		584	445 (23.8)	409 (31.1)
2	53	♀	596	539 (9.8)	549 (2.8)	444		
3	45	♀	479	373 (22.1)	359 (3.8)	512		
4	31	♂	449	306 (31.8)	338 (-10.5)	357	336 (5.9)	299 (22.9)
5	43	♂	460	321 (30.2)	302 (5.9)	410	281 (31.5)	304 (-12.1)
6	66	♀	594	501 (15.7)	x	544	404 (25.7)	317 (21.5)
7	24	♂	492	378 (23.2)	x	379	342 (9.8)	
				Cholestyramine	Cholestyramine + clofibrate		Neomycin	Neomycin + clofibrate
8	34	♂	471	296 (37.2)	241 (18.8)	335	396 (-15.2)	283 (26.7)
9	45	♂	487	345 (29.2)	345 (0.6)	458	341 (25.5)	319 (6.5)
10	33	♂	471	399 (15.6)	430 (-7.8)	439		
11	47	♀	428	306 (29.0)	292 (4.6)	371	300 (19.1)	
12	26	♂	527	371 (29.6)	320 (13.7)	446		
13	58		406					

x = withdrew 1 from treatment because of side-effects.

at 6-week intervals. Audiometry was performed before and after neomycin treatment. At the end of each 6-week treatment period the patients were questioned about problems in connection with their medication and, specifically they were asked about diarrhea, constipation and abdominal pains.

RESULTS

Mean plasma cholesterol values in the different periods of the trial, as well as percentage reduction of cholesterol in relation to the preceding period are presented for each patient in Table I. In no control or treatment period did the mean value differ significantly from the last value measured in that period using the Wilcoxon rank sum test for paired differences. In Table II it can be

seen that neomycin treatment resulted in a median 23% decrease and cholestyramine treatment in a median 27% decrease in plasma cholesterol. These reductions were statistically significant. Adding clofibrate to neomycin and cholestyramine produced a further 5% and 8% median reduction of cholesterol respectively. These reductions were not significant. Using the Mann-Whitney rank sum test for unpaired measurements no significant difference was found between mean cholesterol values during neomycin and cholestyramine treatment. Employing the same test it was found that

Table III. Distribution of 13 patients treated with neomycin and cholestyramine according to degrees of side-effects

Treatment	Side-effects			Wah-draw 1 from treatment	Total
	Toler-None	able	Reduc-tion of dose required		
Neomycin and neomycin + clofibrate	1	4	1	7	13
Cholestyramine and cholestyramine + clofibrate	1	5	3	4	13

Table II. Median reduction of plasma cholesterol in per cent of control or preceding treatment periods

Periods compared	Reduction	%	P
Control 1 and Neomycin	22.6	10	<0.01
Neomycin Neomycin + clofibrate	4.8	6	NS
Control 1 and 2 Cholestyramine	27.3	18	<0.01
Cholestyramine Cholestyramine + clofibrate	8.1	9	0.07

mean cholesterol values in the second control period were significantly lower than in the first ($p < 0.05$).

As can be seen in Table III side-effects were considerable, necessitating withdrawal of 7 patients from neomycin treatment and 4 from cholestyramine treatment. Only patient 5 had no side-effects of neomycin, and only patient 12 no side-effects of cholestyramine. The most common complaint during neomycin treatment was diarrhea and nausea. Cholestyramine caused nausea, meteorism and constipation. Because of the latter 1 patient developed hemorrhoids. Side-effects persisted for the duration of treatment. No side effects could be ascribed to clofibrate.

Triglycerides, which were normal in these patients, were slightly reduced by clofibrate therapy. Phospholipids followed cholesterol variations. Alanine aminotransferase and alkaline phosphatase became moderately elevated while patient 9 was on neomycin. All other variables measured in these 13 patients, including audiometry remained unchanged during the trial.

DISCUSSION

This study demonstrates that substantial and comparable reductions of plasma cholesterol can be achieved by administration of neomycin and cholestyramine. The study also demonstrates that both drugs, and perhaps especially neomycin cause severe discomfort to the majority of patients. Both drugs are thus far from ideal, and on the basis of this study and because of the risk of nerve deafness and renal damage, neomycin would seem to be further from the ideal than cholestyramine, requiring stricter supervision of patients in treatment. Both effects and side-effects of these drugs are, however variable, which for the present emphasizes the importance of an individual approach to drug treatment of these patients.

The statistical non-significance of the further reduction of cholesterol by addition of clofibrate to neomycin and cholestyramine may be due to the smaller number of patients treated and, perhaps, to poorer adherence to the latter two drugs during the last part of the trial. That cholesterol

values during the course of the second control period did not rise to their former levels suggests a prolonged effect of these drugs and demonstrates a shortcoming in the design of this study in that the second control period should have been of longer duration.

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VENTILATORY FUNCTION AND RESPIRATORY LIMITED EXERCISE TOLERANCE IN PULMONARY TUBERCULOSIS

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Abstract. Correlations of respiratory-limited physical working capacity with FEV₁, VC and airways obstruction have been studied in a series of 170 male and 38 female patients with pulmonary tuberculosis. Statistically significant positive correlation was established for exercise tolerance both FEV_{1,0} and VC. Exercise tolerance displayed significant correlation with airways obstruction in such way that the obstructive patients of both sexes showed, on average, a better working capacity than the non-obstructive patients when the material was FEV₁-standardized. This was attributed to probable bronchial relaxation and shift of the respiratory midposition towards higher lung volumes among the obstructive patients in exercise. Significant negative correlation with airways obstruction was established in VC-standardized male group.

The physical fitness of patients with chronic lung diseases depends on a large number of medical, physiological and socioeconomic factors. Exercise tests and pulmonary function studies are largely employed, among other criteria, in the assessment of physical capacity and possible unfitness.

In healthy subjects the performance capacity depends primarily on the evolution of cardiac output. However in patients with chronic lung diseases the breaking point is often achieved before the maximal oxygen uptake is reached. Incapacitating breathlessness is usually the factor which obliges the patient to discontinue further exercise. The basic physiological disorders behind the sensation of breathlessness lack final clarification. Derangements in pulmonary mechanics are obviously largely involved in the genesis of dyspnoea. Ventilatory capacity is regarded as one important factor related to respiratory-limited exercise tolerance in lung diseases (1).

The purpose of the present study was to analyze possible correlations of respiratory-limited

physical working capacity with spirometric values and airways obstruction in pulmonary tuberculosis.

MATERIAL

The material consists of 170 male and 38 female patients treated in Leikoo Hospital in 1961-70 for pulmonary tuberculosis. The criteria used in the selection of the material were: age 20-65 years, reliable spirometry ergometry which had been discontinued on account of breathlessness, no other respiratory diseases which might affect ventilatory function (5), no manifest cardiac or circulatory diseases (5), no previous thoracic operations. Table I gives the physical characteristics of the material.

The distribution of the material according to the ventilatory function (6) was as follows.

Males: normal 22, restrictive impairment 60, obstructive impairment 88.

Females: normal 5, restrictive impairment 14, obstructive impairment 19.

Seventy-eight male and 20 female patients showed positive tuberculin skin test one year before the spirometry.

The mean heart rate at the end of the exercise test was 135 beats/min (S.D. = 20) among male and 141 beats/min (S.D. = 20) among female patients. The mean breathing frequencies were 37 (S.D. = 6) in both sex groups.

The distribution of the material according to the classification of exercise tolerance, given under Methods, is shown in Table II.

METHODS

Spirometry

A Bernacki Spirometer (Kjfa, Sweden) was employed. The patient was seated during the examination. VC, FEV_{1,0} and FVC were recorded. FEV₁ and FVC were measured from the same curve. At least two VC and FES curves were taken; if the values varied, the examination was continued until two approximately similar curves were obtained. The highest VC value was accepted. VC was usually greater than FVC. When it

Table I Physical characteristics of the material

Sex	No.	Mean age (yr)	Height (cm)		Weight (kg)		FEV _{1.0} (%)		VC (%)	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
♂	170	48.9	171.6	6.6	66.1	11.3	56.0	21.1	72.2	18.7
♀	38	43.3	161.0	6.2	60.0	11.6	60.9	21.6	72.4	18.4

Table II Distribution of the exercise tolerance according to the classification used in the series

	Exercise tolerance class									Total
	1	2	3	4	5	6	7	8		
♂	10	26	35	35	28	26	6	4		170
♀	4	9	12	5	7	1	—	—		38

was not, the FVC value was used for VC as well. Use was made of the percentages of the predicted normal FEV and VC (6). The existence of airways obstruction was estimated on the basis of FEV%, the values FEV% predict -13 (males) and FEV% predict -11 (females) were used as lower limits of normal FEV%.

Ergometry

The physical work test was performed on an electrically braked bicycle ergometer (Elems-Schöander Sweden) with the patient in supine position. The heart rate and the breathing frequency were counted and an ECG was taken every 2nd min of the exercise. The test was started at 50 kpm/min and the load was increased in steps of 50 kpm/min up to 1000 kpm/min. Each stage lasted 6 min.

Those patients who discontinued the test on account of breathlessness were selected for the present study. The respiratory-limited physical working capacity illustrated by the work load at breaking point (W_{resp}), was grouped into subcategories in the following way: 1) W_{resp} 50 kpm/min 3 min or less, 2) W_{resp} 250 kpm/min more than 3 min but 4.6 min, 3) W_{resp} 400 kpm/min 3 min or less, etc. (eight classes).

Table III Results of the W_{resp} /FEV model

	Males				Females			
	β	S.E. (β)	R		β	S.E. (β)	R	
FEV _{1.0}	0.057	0.005	10.7	0.76	0.054	0.007	8.09	0.86
Obstruction	0.461	0.225	2.05		1.044	0.294	3.55	
Age	-0.073	0.091	0.80		-0.266	0.078	-3.39	
Age ²	0.00003	0.0009	0.03		0.0022	0.0009	2.43	
Height	0.023	0.014	1.70		-0.014	0.018	-0.76	
Intercept	0.048				8.72			

Statistical methods

The analyses of the relationships of exercise tolerance with airways obstruction, FEV and VC were in the general linear model. Age, age squared and height were added to the model. The form of the model is as follows.

$$E(W_{\text{resp}}) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6$$

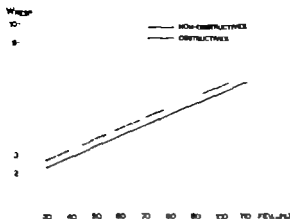
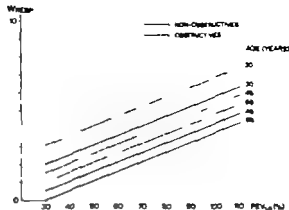
where $E(W_{\text{resp}})$ = expected value of W_{resp} , x_1 = airways obstruction (coded as 0 = non-obstruction, 1 = obstruction), x_2 = FEV (% of predicted), x_3 = age (yr), x_4 = height (cm), $\beta_0, \beta_1, \beta_2, \beta_3, \beta_4, \beta_5$ and β_6 are the estimated coefficients. The same model was used to analyse the correlation of W_{resp} with VC, then x_2 = VC (% of predicted). The analyses were made separately for the two sexes.

RESULTS

The results of the W_{resp} /FEV_{1.0} models are given in Table III and Figs. 1 and 2.

Respiratory-limited exercise tolerance displayed statistically significant positive correlation with FEV_{1.0} ($r \leq 1.96$) in both sexes. Age had also an importance among the female material, even though the FEV_{1.0} values used in the regression analyses were already age- and height-standardized (6). It must, however, be remembered that the standardization had been made in an extrinsic reference population and is thus not necessarily quite relevant in the present material.

It can be seen that the graphs of the obstructive patients are located above those of the non-obstructive patients, the difference being statisti-

Fig. 1 W_{max} as function of $FEV_{0.75}$ (males).Fig. 2 W_{max} as function of $FEV_{0.75}$ at different ages (females).

cally significant. Hence the obstructive tuberculosis group has, on average, a higher performance capacity than the non-obstructive group when the material is standardized in respect to the other parameters (e.g. $FEV_{0.75}$) of the regression model.

Table IV and Figs. 3 and 4 show the results of the W_{max}/VC model. Here too, a close (positive) correlation is established between exercise tolerance and VC. Significant (negative) correlation with airways obstruction is revealed in the male group.

DISCUSSION

A large group of different respiratory factors are involved in the evolution of physical working capacity among lung patients. Thus a proper evaluation of ventilation as a limiting factor meets difficulties. Dynamic spirometry is, however regarded as an informative examination in appreciating the exercise tolerance (1, 2). Registration of flow-volume relationships at rest and during exercise, and maximum expiratory flow-volume

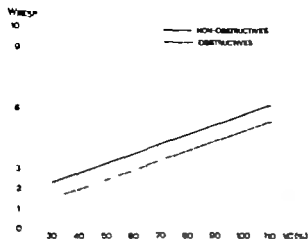
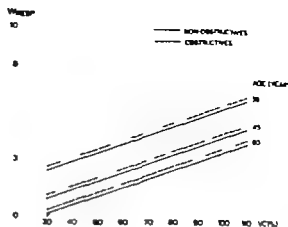
curves, has recently offered a useful approach to the ventilatory limitation of physical working capacity.

Simonsson et al. (7) found $FEV_{0.75}$ to correlate significantly with maximal work load in their series of patients with chronic bronchitis. The maximal work did not, however illustrate purely respiratory exercise tolerance, as the material included also patients who had discontinued the exercise test because of circulatory symptoms. In the present series the positive correlation between $FEV_{0.75}$ and exercise tolerance was confirmed in tuberculous patients. Furthermore VC correlated significantly with physical performance capacity.

A significant negative correlation reveals between airways obstruction and exercise tolerance when the material is $FEV_{0.75}$ -standardized. This finding apparently indicates that the obstructive patients can adapt their ventilation in exercise in a different way than the non-obstructive patients. Several mechanisms of adaptation could explain this phenomenon. Shift of ventilatory midposition toward higher lung volumes, where the airways

Table IV Results of the W_{max}/VC model

	Males				Females			
	β	S.E. (β)	R		β	S.E. (β)	R	
VC	0.047	0.005	9.99	0.73	0.046	0.009	5.93	0.80
Obstruction	-0.762	0.184	-4.14		0.141	0.294	0.48	
Age	-0.153	0.093	-1.61		-0.213	0.094	-2.27	
Age ^a	0.0008	0.0010	0.78		0.0015	0.0011	1.42	
Height	0.024	0.014	1.85		-0.0014	0.0221	-0.06	
Intercept	1.99				6.11			

Fig 3 W_{resp} as function of VC (males).Fig 4 W_{resp} as function of VC at different ages (females).

are wider allows a higher maximum expiratory flow (3-4). Shortening of the time of inspiratory phase also provides an opportunity of increasing the breathing capacity during exercise. Finally bronchodilation during exercise has been proposed as one mechanism of adaptation in obstructive lung patients (3).

The positive correlation of airways obstruction with respiratory-limited exercise tolerance in VC standardized material seems natural. Ventilatory capacity illustrated by $FEV_{1.0}$ is lower in the obstructive group when the material is VC-standardized.

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THE SIMULATION OF THE ELIMINATION PROCESS FOR A SUBSTANCE INJECTED INTO THE BLOOD STREAM

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Abstract. The elimination process of substances from the blood stream has been analysed in physical and biochemical terms taking into consideration both the enzyme-substrate reaction, the possibility of phagocytosis or diffusion and the uneven distribution of the receptor sites in the body. The elimination process as expressed in mathematical terms and simulated in computer. The elimination curve was studied by changes of different parameters in the equations. It was found that at high substrate concentrations, when the enzyme capacities were saturated, there was a linear elimination rate. The slope of that part of the curve was depending on the enzyme activities. At low concentration the elimination was exponential. The slope of this part of the curve was influenced by changes of the blood flow to the receptor sites. The phagocytotic model was more effective than the enzymatic models under similar conditions, fact which may be of importance in biological evolution with competition between different systems.

The method of injecting a substance into the blood stream and the subsequent sampling of blood for analysis has been used in a vast number of studies in order to get information about its metabolism. The method also implies plotting of the analytical results on a graph and then calculating the slope of a straight line between points on the graph.

In order to obtain straight lines the observations are analysed in different ways and the calculated slopes are expressed by a constant, a k -value. Usually a high numerical k -value is interpreted as a fast metabolism and a low value may indicate a pathological state. This can be exemplified by the low k -value obtained in the i.v. glucose tolerance test in diabetic patients and the value for bromsulphthalein elimination in cirrhotic patients. Furthermore the k -values obtained are often correlated to the blood concen-

tration of different substances. In that way information is collected about metabolic physiology and pathogenesis.

Only a few studies are concerned with the biochemical-physical reality reflected in a k -value so obtained. It is most relevant to raise the question, what physical or biochemical processes are involved when a k -value is abnormal?

This report has analysed some k values against a background concerned with the elimination from blood of i.v. injected substances such as chylomicrons or fat emulsions for parenteral nutrition (2, 3, 4, 6, 7). From a kinetic point of view these exogenous lipids have some advantages over many other substances: firstly they are particulate and seem to be distributed only in the blood circulation (5, 6, 7), and secondly their metabolic enzyme, lipoprotein lipase, seems to be located very close to the vessel wall (10). These two features reduce the number of assumptions necessary for the building of a model illustrating the elimination process. Such a model has previously been suggested and simulated by Simpson-Morgan (12).

THE MODELS

The blood stream was considered as one pool circulating in the body. At certain sites in the walls of the blood vessels there are receptors or pores for elimination of the injected substance. The property of the contact between the injected substance and the receptor site is considered to be of three different types, here defined as models 1, 2 and 3 and schematically illustrated in Fig. 1.

List of symbols

S = substrate, E = enzyme, P = product formed in the enzyme-substrate reaction, SE or ES = enzyme-substrate com-

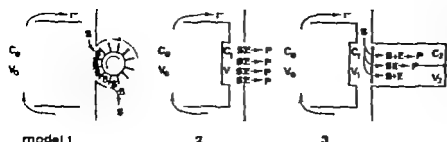


Fig. 1 Schematic illustrations of models 1, 2 and 3. They illustrate the uneven distribution in the circulation of three different types of receptors for a substance (S) injected into the circulation. Symbols are the same as symbols in the text.

plex, V = volume of blood pool, V = volume of receptor area, V = volume of post-dialysis receptor area, C_0 = substrate concentration in circulation, C = substrate concentration in receptor area, C = substrate concentration after dialysis, $C = \dot{C}$, \dot{C} = rate of change of respective concentration, C_{ES} = concentration of enzyme-substrate complex, C = concentration of free enzyme, C_{ET} = total enzyme concentration, R = regeneration rate of receptor site, v = blood flow rate to receptor site, k = reaction rate constant, $E + S \rightleftharpoons ES$, k_{-1} = dissociation rate constant, $ES \rightarrow E + P$, k = reaction rate constant, $ES \rightarrow E + P$, k = dialysis constant.

Model 1

The receptor site in the vessel wall was considered to be "sticky" for the injected substance. This means that when the substance comes into contact with the site it sticks to the vessel wall and is thus taken away from the circulation. The substance is then transported away say by some kind of pinocytosis. The regeneration of the sticky sites is considered as a continuous process (illustrated in Fig. 1 model 1 by a cogwheel rotating at constant rate R).

Model 2

The receptor site in the vessel wall was considered to be an enzymatic quality which transforms the injected substance according to the well known reaction.



where E , S , ES and P are an enzyme, the substrate, enzyme-substrate complex and product, respectively and k_1 , k_{-1} and k are rate constants (Fig. 1 model 2).

Model 3

The receptor site in the vessel wall was considered to be a semipermeable membrane for the injected substance. On the other side of the membrane the substance comes into contact with an enzyme with the same properties as in model 1 (Fig. 1 model 3).

This simplified law of the elimination process illustrates three important real phenomena which are relevant for the elimination of substance from the blood stream: (a) the flow of blood to the receptor site (b) the passage through the vessel wall, (c) the enzymatic reaction.

Disturbances in the elimination process as reflected by abnormal k -value may be confined to any one or combinations of these three factors. It is therefore of interest

to simulate such disturbances and to map their characteristic features as seen in the curve for elimination from the blood stream of the injected substance.

MATHEMATICS

The elimination of the substance in the three models is mathematically expressed in the equations below. The symbols in these equations are given above.

The following assumptions are considered to be valid. The product formed in the enzymatic reaction is rapidly taken away and no product inhibition occurs. The mixing in V_0 , V_p , and V is instantaneous.

Model 1

When the rate of regeneration (R) of the receptor site is faster than the transport function ($v \cdot C_0$) of the substance, then the following equation is valid for the rate of elimination:

$$C = -\frac{R}{v} C_0 \quad (2)$$

When the transport function ($v \cdot C_0$) is faster than R then the following equation applies:

$$C_0 = -\frac{R}{v} \quad (3)$$

Equations 2 and 3 may be combined to describe C at all concentrations C_0 as follows:

$$C_0 = -\frac{R}{v} C_0 \quad (C_0 < R/v) \quad (4)$$

$$= -\frac{R}{v} \quad (C_0 > R/v)$$

Model

The reaction represented by equation 1 reflects the statistical uncertainty associated with a real process. There is no longer a sharp transition between two elimination rate equations as in model 1 but rather a varying probability of free enzyme molecules combining with, and remaining combined with, substrate molecules. Asymptotically the two models exhibit identical characteristics.

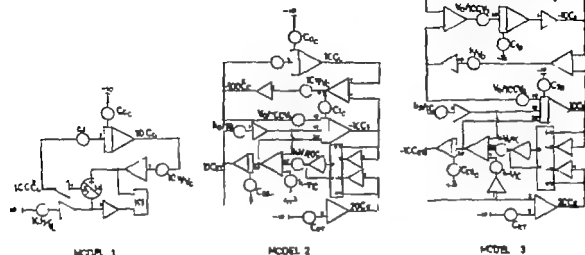
For the blood pool

$$C_0 = -\frac{1}{V} (C - C_0) \quad (5)$$

For the receptor area

$$C = \frac{R}{v} (C_0 - C) - k \{ C C_E + k_{-1} C_{ES} \} \quad (6)$$

Fig. 2 Patching diagram for an analogue computer of the equations describing the function of the models illustrated in Fig. 1



$$C = -k_v C C_E + (k_r + k_d) C_{ES} \quad (7)$$

$$C_{ES} = C_{ET} - C_E \quad (8)$$

Model 3

When semipermeable membrane is interposed between the receptor area and the area in which the enzyme acts, the transport of substrate to the enzyme is impeded in accordance with the following equations:

For the blood pool

$$C_b = -\frac{1}{V} (C_b - C_1) \quad (9)$$

For the receptor area

$$C_r = \frac{1}{V} (C_b - C_1) - \frac{k}{V} (C_1 - C_2) \quad (10)$$

For the postdialysis receptor area

$$C_1 = \frac{k}{V} (C_1 - C_2) - k_1 C_1 C_E + k_{-1} V C_{ES} \quad (11)$$

$$C_E = -k_1 C C (k_r + k_d) C_{ES} \quad (12)$$

$$C_{ES} = C_{ET} - C \quad (13)$$

PROCEDURE OF ANALYSIS

The equations for the three models were scaled and patched on an analogue computer (TR-48) using the patching diagrams shown in Fig. 2. The time scaling as shown was 1 sec/min, and the integrator gains were all increased by factor of 10 giving an overall time scale 600 times faster than real time. The results were recorded on Hewlett-Packard XY recorder and Brush 8-channel strip-chart recorder.

RESULTS

Fig. 3a shows an example of the elimination from the circulation (V_b) of different initial amounts of the injected substance in model 3. The corresponding curves in models 1 and 2 were in principle very similar.

Results obtained in vivo from one dog given single I. injections of different amounts of triglycerides (8) are shown in Fig. 3b and c. The pattern of the changes in Fig. 3a is very similar to those in Fig. 3b.

Some characteristics are obvious in these curves. All curves are parallel. Thus the shape and slopes are independent of the injected amount. At high concentrations the curves are arithmetically linear and at low concentrations semilogarithmically linear (Fig. 3c).

The slopes of these curves thus correspond to the clinically used k -values mentioned in the introduction. These k -values correspond to C_b , which is the rate of change of concentration in this report. The analysis has therefore focused on factors influencing the rate of change of concentration (C_b). Fig. 4 was obtained by recording C_b versus C_b . This figure gives the characteristics in the three models when all com-

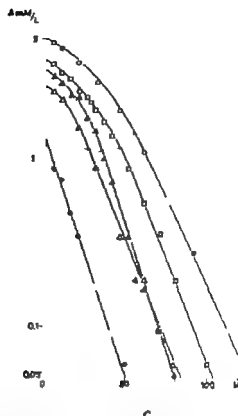
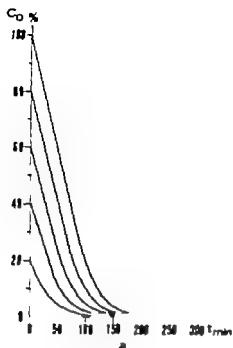
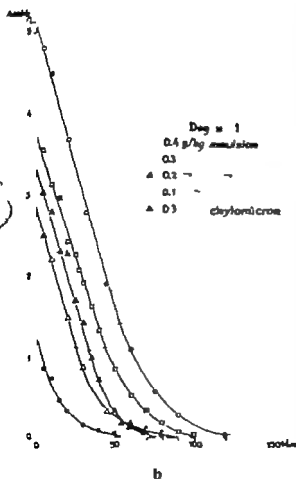


Fig. 3 The elimination of different doses of a substance injected into the circulation of model 3 in linear scale. Percentage of the initial concentration remaining in the circulation is plotted versus time (a). The elimination is two of different doses of a fat emulsion and chylomicrons injected into the blood stream of dog. The triglyceride concentration versus time is arithmetic (b) and semilogarithmic (c) scales.



parable parameters were equal. Common to all three curves is that at high and low C_0 the curves are all identical. It is also obvious that C_0 is dependent on C_0 only at low concentrations.

The difference between model 1 and the enzymatic models 2 and 3 was that the area under the curves for the enzymatic models is smaller than in model 1. This area is a reciprocal measure of the time of elimination. Thus the elimination time for a given amount of substance was shortest in model 1 which is therefore the most effective of the three.

Fig. 5 illustrates curves obtained by changing only v in model 1 (or v/f_0 , the fractional flow). The effect was a change only in the slope of the curve at low concentrations (the exponential linear part of the elimination curve versus time see Fig. 3c). At high C_0 the curve did not change

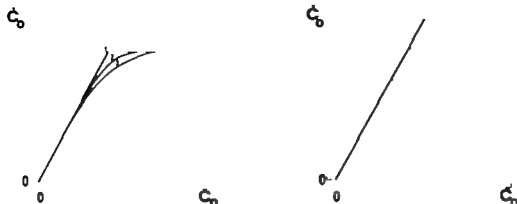


Fig. 4. C_0 at different C_0 injected into the circulation in the three different models shown in Fig. 1. The three curves were obtained from models 1, 2 and 3, respectively. All comparable parameters were the same.

The influence of variations only in R and C_{mf} in models 1 and 2 is shown in Fig. 6a and b. It is seen that these variables only influence the maximum C_0 values (at high C_0). This corresponds to the slopes of the arithmetic linear part of the curves in Fig. 3a and b.

Variations of only one of the rate constants k_1 and k_2 in the enzymatic reactions, keeping all other parameters constant, influenced the curves as seen from Figs. 7a and b and 6b. The principle of these variations was similar in models 2 and 3.

Variation of k_1 (Fig. 7a) influenced the curve considerably both the level at high C_0 and the slope of the curve at low C_0 .

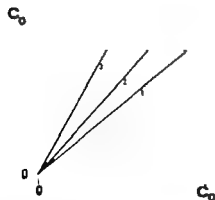


Fig. 5. C_0 at different C_0 injected into the circulation of model 1 (see text and Fig. 1). The different curves (1, 2, 3) denote the effect of different k_1 and k_2 values, all other parameters being kept constant.

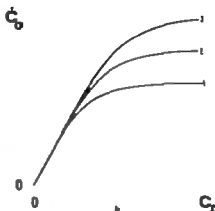


Fig. 6. C_0 at different C_0 injected into the circulation of models 1 (a) and 2 (b). Numerals 1, 2, and 3 denote curves obtained by changing only the capacity R and C_{mf} respectively. All other parameters were constant.

Variation of k_2 (Fig. 7b) influenced the curve only to a small degree in all intervals studied in the present scaling.

Comparisons between models 1 and 3 revealed that the only difference, the dialysing membrane, added a retarding effect to the flow factor (v/V_0).

The curves (C versus C_0) were identical in models 2 and 3 if the dialysing constant, k was set at an appropriate level and all other parameters were kept at the same values.

DISCUSSION

It is obvious from the analysis that the clinically used k -value, expressing the elimination of an injected substance from the blood stream, may be an indicator for several physical-biochemical

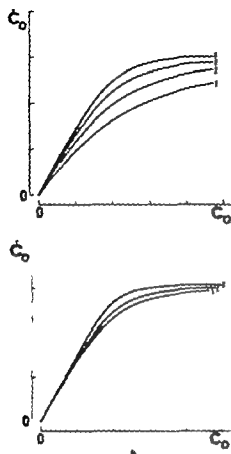


Fig. 7 C at different C injected into the circulation of model (see text and Fig. 1). Numbers 1, 2 and 3 and 4 denote different curves obtained by changing the value of the rate constants (k_1 in a , and k_2 in b) in the enzymatic reaction (eq. 1).

ies. In the models used here the conditions were based on the reasonable assumption of an uniform distribution of the site of elimination in the space occupied by the injected substance. Thus the flow to the active site is one factor of importance for the elimination. A second factor was the capacity of the active site. These two factors are rate-determining but at different concentrations. A third factor was the rate constants in the enzyme-substrate reactions in models 2 and 3. These factors determined the shape of the curves at a zone between high and low substrate concentrations.

The most effective elimination process was obtained in model 1 by assuming "stickiness". In this model the intermediate zone was absent. The enzymatic models thus had a lower effectiveness, which is clear if one realizes the reversible reac-

tion and the enzyme-substrate complex formation.

The difference in effectiveness between the three models (see Fig. 4) might be of importance in a competition during the evolution of biological systems.

It was possible to characterize the first two factors by the shape of the elimination curve. If the elimination curve was arithmetically linear the capacity factor was rate-determining, and if the curve was semilogarithmically linear the flow factor was rate-determining. In the intermediate zone the curve was linear neither arithmetically nor semilogarithmically.

Thus an abnormal k -value obtained from a curve appearing as a straight line semilogarithmically may indicate a disturbance either in the flow or in the permeability factors and not in the enzymatic capacity. Changes in the rate constant k_1 in the enzymatic reaction, could disturb this rather clear picture in models 2 and 3 which fact is interesting. It could mean that the effectiveness of such a system could be regulated by two means: a change of such a physical factor as the blood flow could be compensated for by a change in a biochemical rate constant. Whether such a feed-back system exists is not known to us.

This analysis implies for its practical application that it is necessary to determine the concentrations at very close intervals to determine the curve in the intermediate zone, where the curve is not linear.

Another application of the analysis will be for artificial organs in contact with the blood stream. These units usually contain a dialyzing membrane with blood on one side and sometimes an enzyme or a resin for ion exchange on the other side to correct metabolic errors. It is possible to calculate the theoretical efficiency of such an apparatus from the models presented.

Garrett (1) pointed to the possibility of characterizing the receptor site for drugs from the nature of the time courses of blood levels. In this study the kinetics were of a different order at high and low concentrations. Variable kinetics depending on the concentration level have been discussed and described during recent years, and different biological models have been presented (9, 11, 14, 15).

Most information concerning enzyme-substrate

kinetics is obtained from *in vitro* studies with even distribution of enzyme and substrate in the test tube. *In vivo*, on the other hand, there exists an uneven distribution of enzymes and their substrates. They are very often separated by a membrane, as in the design of this study

ACKNOWLEDGEMENTS

Supported by the Swedish Medical Research Council (Grant No. B-Lix 2057-04) and Anderssonsfonden, Karolinska Institute.

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TRYPTOPHAN MALABSORPTION IN LEVODOPA TREATED PARKINSONIAN PATIENTS

Effect of Tryptophan on Mental Disturbances

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Abstract. Subnormal serum tryptophan (Try) response has been measured in parkinsonian patients receiving oral Try loads while under levodopa treatment, indicating malabsorption of Try in the gut. Other amino acids (phenylalanine, tyrosine and glutamic acid) also depressed the absorption of Try. If levodopa was excluded during Try loading, nearly normal serum Try values were measured. Similar tests in a normal subject revealed normal absorption of Try. An explanation of these observations is discussed and an insufficiency (degeneration?) of amino acid carriers in the intestinal mucosa, and possibly also in other membranes, is suggested in parkinsonian patients. Mental disturbances were observed in cases with extremely low serum Try values and were presumed to be correlated with an insufficient synthesis of 5-hydroxytryptamine (5-HT) in the brain secondary to decreased levels of Try. In two cases considerable improvement of mental symptoms as noted after oral Try medication, and in one case regression of the ability to absorb Try during levodopa treatment was noted. A prophylactic Try or protein treatment during levodopa treatment was recommended.

In a previous paper (26) attention was called to the possibility that the treatment of parkinsonian patients with levodopa might produce a tryptophan (Try) deficiency from competition between levodopa and Try. It was also suggested that an interference with Try could be responsible for the mental depression and other side-effects observed during levodopa treatment. Based upon this presumption, a combined levodopa and Try (L Try) treatment was recommended and shown to be effective in a patient who had suffered severe mental deterioration during levodopa treatment.

In the present study serum Try levels were

measured after oral Try loads, and data are presented demonstrating a competition between levodopa and Try at the levels of the intestinal absorption of Try. The results of the experiments are presented and discussed with special regard to mental disturbances. In one further patient L Try treatment resulted in a considerable improvement of the mental condition.

This investigation was partly undertaken to determine whether the low serum Try values measured in carcinoma patients with liver metastases and mental disturbances under conditions of fasting and Try loading were pathognomonic for this disease (24). Therefore patients within the same age group with other diseases but also displaying mental symptoms (Parkinsonism and senile dementia) were investigated. The intention was also to determine whether Try medication, which was found to alleviate the mental symptoms in carcinoma patients (24), would also be beneficial in the treatment of mental disturbances associated with Parkinsonism and senile dementia.

METHODS

Try in serum was analyzed according to Denckis and Dewey (11), in urine according to Lehmann (25), 5-HT in whole blood according to Halsey and Moos (19), and 5-HIAA (5-hydroxyindoleacetic acid) in urine according to Contractor (9) (modified). Capillary blood (finger tip) for Try analysis in serum was collected before and 1, 3, 4, 6 and 8 hours after the Try loads. In some experiments blood samples were taken only before and 4 and 6 hours after the loads. Urines were collected during the night before Try loading and over an 8-hour period after the loads.

Oral Try loads, 100 mg/kg, were administered in 300 ml

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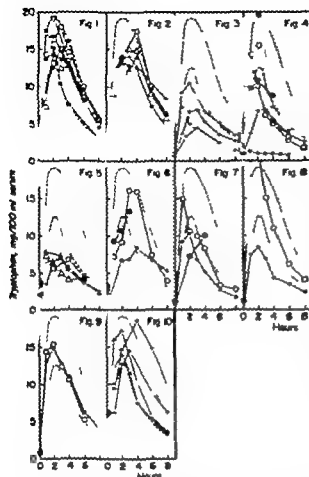


Fig. 1-10 Try in serum after oral Try loads, 100 mg/kg. When not otherwise stated the loads were administered at 8 a.m. in fasting condition. +---+ ordinary load before levodopa; ●—● Try+levodopa during the load; ○—○ levodopa excluded during the load; ●—● levodopa excluded (evening load); A—A Try+Tyr load; Δ—Δ, Try+Phe load; □—□ Try+Gln normal range.

plum milk at 8 a.m. to patient fasted overnight. Levodopa (Doparal[®] Acta Söderström, Sweden, a slow release tablet of 0.4 g), when given together with the Try load, varied from 0-10 g and was administered at 8 a.m. and at noon. When given routinely third dose was given at 4 p.m. When L-tyrosine (Tyr), L-phenylalanine (Phe) or L-glutamic acid (Gln) respectively were given together with the Try load, a dose of 50 mg/kg, they were suspended in the milk together with Try. A light meal was taken at 1 p.m. (4 pieces of bread and butter tea or coffee). L-Tyr was obtained from Aynamoto Corp., Tokyo, Japan; the other amino acids were obtained from Merck. When Try was administered routinely without simultaneous levodopa treatment, 5 g was given 3 times daily with the meals. When given during levodopa therapy the Try was administered in single dose of 3-5 g in the evening 3 hours after the last levodopa dose (see later). The Try powder was suspended with 1-2 teaspoons of sugar in 100 ml sour milk. It given intravenously 5 g

Fig. 1 Try loads in 8 normal subjects, 41-45 years (mean 60). The symbols in this Figure are not the same as in the other Figures. In Fig. 1 they only designate different subjects.

Fig. 2 Normal subject, man, 72 years. Ordinary load and Try loads combined with levodopa (0.4 g+0.4 g). Tyr, Phe and Gln. The latter loads 50 mg/kg.

Fig. 3 Cases 1, 2 and 3 Try loads before levodopa. Case 1 after LV and oral Try treatment.

Fig. 4 Case 2 Try+levodopa (0.6 g+0.6 g) and Try with levodopa excluded. After 3 months treatment with Try and protein and resumption of levodopa treatment the Try loads were repeated. A remarkable increase in the absorption of Try was observed both with Try levodopa, 0.6 g+0.6 g (●—●) and with levodopa excluded (○—○). The evening load was administered 1 hour after the last levodopa dose (0.6 g).

Fig. 5 Case 3 Try loads combined with loads of Tyr, Phe and Gln.

Fig. 6 Case 4 Try+levodopa (0.6 g+0.6 g) and Try with levodopa excluded. Evening load 3 hours after the last levodopa dose (0.6 g).

Fig. 7 Case 5 The same loads as in Fig. 6, levodopa 0.2 g+0.2 g. Evening load 45 min after the last levodopa dose (0.2 g).

Fig. 8 Case 6. The same loads as in Fig. 7 levodopa 0.2 g+0.2 g. No evening load.

Fig. 9 Case 7 The same loads as in Fig. 8, levodopa 1 g+1 g.

Fig. 10 Try loads in 4 patients with senile dementia.

were dissolved with 244 ml 1 N HCl, diluted to 50 ml with sterile water and the solution was sterilized by filtering through a Seitz filter into a sterile ampoule. The pH was 1.2-1.4. The ampoule was stored at room temperature usually not more than 3 days. If precipitates occurred, the ampoule was warmed in hot water. Before use the ampoule was emptied into 1 l intravenous amino acid infusion (mixture of crystalline L-amino acids corresponding to 70 g protein containing all essential amino acids, Astra Söderström, Sweden) or as an alternate procedure, into 1 l Aminosol 5% glucose for infusion (Carmen hydrolysate corresponding to 38 g protein, Vitrum, Stockholm, Sweden). The pH in the final infusion solution was approximately 3.8. The infusion time was 3-7 hours. Usually the infusion was repeated on 2-3 consecutive days. When oral Try treatment, given in the evening, was exchanged for a protein-enriched meal after improvement of the mental symptoms, this consisted of 20 g (2 tablespoons) of a casein preparation, "3 61" containing 67% casein (Carmen Stockholm, Sweden), 2 teaspoons sugar and 1 teaspoon cocoa powder suspended in 200-300 ml milk.

MATERIAL AND ANALYSES

Normal subjects

Try loads were given to 8 normal subjects, aged 45-79 years (mean 60) 4 men and 4 women. The results are

illustrated in Fig. 1. In one subject, 72-year-old man, Try loads were combined with loads of levodopa (0.4 + 0.4 g), Tyr, Phe and Gln, respectively (Fig. 2).

Parkinsonian patients

Seven parkinsonian patients, all from Högbo Hospital (with exception of case 1, were analysed. All patients had displayed parkinsonian symptoms, akathisia, rigidity and tremor for 5-8 years. As no correlation was found between localization and severity of symptoms and the response to levodopa treatment, no detailed description of the symptoms is presented. Attention has, however, been paid to mental symptoms and behaviour as these parameters were influenced by Try treatment in some patients. Of the seven parkinsonian patients cases 1, 2 and 3 had been on levodopa treatment before the experiments with Try loads were started, but levodopa was withdrawn due to side-effects. Several months later these patients were tested with Try loads. These loads have been denoted as "before levodopa" (see the Figures), as levodopa was later instituted again and the foregoing treatment was considered to have had no influence upon their general condition, which had stabilized. Case 1 has been reported upon previously (1, 26). All other patients were on levodopa treatment when the experimental trials with Try loads were started and were therefore only tested with "Try+levodopa" and the "levodopa excluded" during the load. The different loads were given at intervals of at least one week in order to exclude the possibility that Try pyrolysis activation by load might interfere with the results of following load.

Case reports

Case 1 A man, 50 years old, referred to above (case 1 in ref. 1) (26). The patient deteriorated mentally during levodopa treatment and was in stuporous state during several weeks after withdrawal of levodopa. A Try load "before levodopa" (Fig. 3) showed very low serum Try values. After oral Try therapy supplemented by homogenized raw liver and meat by stomach tube (300 g of each daily for 5 days), the patient partially recovered. The raw liver was found to be more effective than 6 g L-Try daily. A Try load after this treatment revealed slightly higher serum Try values (Fig. 3).

Case 2. A woman, 66 years old. When admitted (May 1970) the patient showed poor local orientation with some but better orientation to time. She could feed herself and rise from her bed and walk with some help. Some improvement was noted after anticholinergic treatment. On June 1, 1970 levodopa treatment as instituted and increased to 3 g/day. On June 29 the dose was diminished due to dyskinesia (mouth) and mental confusion. As the latter increased continuously levodopa was withdrawn on July 22. A psychiatric consultant (Oct. 15) considered the patient of case of advanced dementia, displaying symptoms similar to those of senile dementia. It was now difficult to establish contact with the patient, she could only express a few words and was difficult to understand. She could not feed herself, nor stand alone. After 2 months in this condition an oral Try load as administered (Fig. 3, "before levodopa"), showing half

the normal serum Try values. Fasting Try value 0.45 mg% (normal 0.9-1.8 mg%). 1. Try treatment (see Methods) was instituted (Oct. 6). Four hours after the commencement of the infusion (2 g L-Try) the patient reacted by following the personnel in the room with her eyes, answered questions and spoke a few words spontaneously. A second 1. infusion was given during the night followed by oral administration of 1 g Try 3 times daily in connection with meals. To further infusions were given later with continuous improvement in mental condition and physical movements. She could now feed herself. On Nov 3 levodopa was instituted again together with continued oral Try medication. On Jan. 20, 1971— months after the beginning of the levodopa treatment—a new Try load was administered together with 0.6 g levodopa as 8 a.m. and at noon. There was no increase in the serum Try values after the load (Fig. 4 "Try-levodopa"). One week later new Try load as administered in which levodopa was excluded during the load. An unexpectedly high Try absorption was found, serum values being close to the normal response (Fig. 4 "levodopa excluded"). Try was thereafter administered in the evening instead of in combination with levodopa. On May 7 further Try load was given 3 hours after the last levodopa dose (0.8 g) in order to see whether this interval is sufficient to prevent inhibition of the absorption of Try. Serum values at the lower range of normal were found (Fig. 4, "levodopa excluded, evening load"). Three further loads combined with Try Phe and Gln respectively (Fig. 5) disclosed low serum Try values. On June 16 the administration of Try was stopped and exchanged for protein-enriched meal (see Methods) in the evening. In Sept. 2 g Gln was added to the evening meal. In Oct. the Try loads "Try+levodopa" and "levodopa excluded" were repeated. A considerable increase in the ability to absorb Try was found (Fig. 4).

After few months treatment with Try remarkable improvement was seen in the patient's mental and general condition. She increased in weight from 46 kg in Oct. 1970 to 72 kg in March 1971. She spoke more spontaneously, laughed, developed more facial movements and read the newspapers daily. She could participate in occupational and physiotherapeutic exercises, walking with some help. This improvement slowly continued also after the Try treatment was exchanged for the protein meal with Gln in the evening. Her voice is, however, not so good and she has difficulties in speaking.

Case 3 A man, 80 years old. He displayed difficulties in walking, mental depression and spontaneous burning into tears. He was treated with levodopa for 3 weeks but it had to be withdrawn because of hypotension, depression and cervical pain. One year later Try load disclosed subnormal serum Try values (Fig. 3). An attempt was now started with levodopa again, the time after oral pretreatment for a week with 1 g Try 3 times daily. After a few days the patient displayed the same side effects as before, including the peculiar cervical pains, and levodopa was therefore withdrawn.

Case 4 A man, 74 years old. During the past 5 years the patient had suffered from dizziness and difficulties in walking. Two years before admission he had slight commotio cerebri. Thereafter difficulties developed in

moving and speaking. On admission he demonstrated truncal rigidity cogwheel phenomenon in the legs, aphonia and difficulties in getting out of bed. Anticholinergic treatment was withdrawn due to hallucinations. However mental deterioration continued and it became difficult to establish contact with the patient. Levodopa was instituted, with some improvement in motility but not mentally. A Try load (Try+levodopa (0.6 g+0.8 g) showed half the normal serum Try values (Fig. 6). Two other loads with "levodopa excluded" one in the morning and one in the evening 3 hours after the last levodopa dose, revealed normal absorption (Fig. 6). The patient was treated with L-Try without any effect mentally.

Case 5 A woman, 86 years old. On admission the patient was unable to rise from her bed and to walk without assistance. Mental functions were severely impaired. It was difficult to communicate with the patient, partly due to deafness. Levodopa treatment induced improvement in motility. A Try+levodopa load revealed serum Try values below the normal limits (Fig. 7). When levodopa was excluded during the load, the maximum value after one hour was normal, followed by a steep fall to slightly subnormal values. The corresponding load in the evening, 45 min after the last levodopa dose, revealed delayed absorption, presumably due to inhibition by levodopa (Fig. 7). L-Try treatment administered as in case 2 brought no significant improvement in mental activity hearing or speaking.

Case 6 A woman, 73 years old. Beside the usual parkinsonian symptoms she suffered mental depression. On admission she could walk with some help. Levodopa was instituted, with improvement in general condition, mentally as well as in locomotion. Hypotension was noted when levodopa was increased to about 0.6 g/day. A Try+levodopa load showed subnormal serum Try values. A similar load with "levodopa excluded" resulted in serum values slightly higher than normal (Fig. 8).

Case 7 A woman, 70 years old. Ten years previously she had had epileptic seizures and weakness in the left leg. A lumbar puncture revealed no spinal tumour. EEG showed lateral nonspecific changes. Rigidity developed in the left of the body and, later on, also in the right arm. She noticed periods of mental depression but was otherwise intellectually normal. She had received levodopa treatment during the past 3 years with good effect on motility. A Try load during levodopa showed completely normal serum Try values in spite of taking 1 g/day at 8 a.m. and 1 p.m. A identical cure was found when levodopa was excluded during the load (Fig. 9).

Patients with dementia praecox

Four patients, 1 man and 3 women, 74, 76, 83 and 84 years of age were tested with Try loads. Serum Try values were within the normal range in the other patients. One patient who had a somewhat delayed absorption (Fig. 10).

Special analyses

5-HIT in whole blood was found to be subnormal in case 1 (0.04 µg/ml) (normal 0.9-0.2 µg/ml) and in case 2 (0.07 µg/ml) but normal in the other patients. Fasting Try values in serum were also extremely low in case 1 (0.46-0.65 mg%) and in case 2 (0.45 mg%) (normal 0.9-

3.8 mg%). In cases 5 and 6 the serum Try level was in the lower range 0.8 and 0.9 mg% respectively but normal in the other patients. Excretion of Try into the urine in 3 normal subjects varied in the nocturnal urines from 20-30 mg/g creatinine, in the Try load urines from 66-128 mg/g creatinine. In cases 3, 5 and 6 the night urine values varied from 1.5 to 4 mg/g creatinine and the load urines from 2.13-14.3 mg/g creatinine. In case 4 the values were 19.6 and 20 mg respectively and, in case 7 7.4 and 63 mg respectively. In cases 3, 5 and 6 the urines were heavily infected by *B. Proteus*. 5-HIAA was analysed in the same urines as Try and varied from 4.34 to 8.9 mg/g creatinine in the night urines, being 1 to 1.8 times higher in the individual Try load urine which is within the normal limits (in case 4 it was, however, 2.3 times higher).

RESULTS AND DISCUSSION

The present investigation has revealed that:

1) Certain parkinsonian patients (cases 1 and 2) displayed subnormal fasting serum Try values before levodopa treatment. After a Try load cases 1, 2 and 3 showed a subnormal response to the load.

2) If a Try load was combined with a levodopa load most parkinsonian patients (all except case 7) also showed a subnormal response to the Try load in spite of a normal response when levodopa was excluded from the load (cases 4, 5 and 6).

3) When a Try load was combined with a Try-Phe or Glu load, respectively a normal subject reacted with a normal Try response but a parkinsonian patient (case 2) with a subnormal load.

Several factors have to be taken into consideration in order to interpret the serum Try values after Try loading. The primary and most important factor is the intestinal absorption of Try. The fate of the absorbed Try is influenced by the following factors: excretion into the urine distribution among inter- and intracellular compartments, metabolism and participation in the synthesis of proteins. Among the latter factors the excretion into the urine is most easily analysed. This was done in 5 of the parkinsonian patients and in 3 normal subjects. It was expected that in the patients with the low Try serum values a diminished excretion would occur as compared with the patients with high serum values. This was found to be the case but the results are invalidated by the presence of bacilluria (*B. coli*, *Proteus*, enterococci) metabolizing Try both *in vivo* in the bladder and *in vitro* in the specimen bottles. This may explain the extremely low values

of Try found both before and after the Try loads in such infected urines. Thus no conclusions could be drawn about the real excretion of Try. Case 7 had no bacteriuria, displayed normal serum Try values after a Try load both with and without levodopa, and excreted normal amounts of Try into the urine.

In order to elucidate the effect of Try metabolism and protein synthesis on serum Try values, experiments with a Try isotope are necessary. Such experiments would involve quite elaborate analyses such as the determination of Try and its many metabolites in urine, faeces and blood as well as the turnover of Try in liver and muscle biopsies. As little is known about the factors influencing serum Try values, it was preferred to consider the intestinal absorption of Try as the predominant determinant of serum Try values measured. After making this assumption, one must consider the possible factors causing a decreased absorption of Try before as well as during levodopa treatment. At least 4 such factors may be considered. 1) A general degeneration of the cells may have taken place in the mucosa of the small intestines where the amino acids are absorbed (in analogy with the age degeneration of the HCl-producing cells in the stomach). 2) An isolated degeneration of the amino acid carriers in the mucosa responsible for the transport of amino acids through the mucosa to blood and lymph may have occurred. 3) A deficiency of different factors necessary for the activity of the carriers may be responsible. 4) The presence of an inhibitory factor in the mucosa capable of decreasing the absorption of Try should be considered.

It is generally accepted that the monoamine monocarboxy acids are transported by the same carriers (8, 29-32). A competition for the carriers between the aromatic amino acids in the gut has been shown *in vitro* (27) and in the brain *in vivo* (18). High plasma levels of Phe inhibit the transport of 5-HTP into the CSF of dogs (34) and inhibition of the Phe absorption by Try has been demonstrated *in vitro* (37). Likewise the transport of 5-HTP into brain slices is inhibited by other amino acids including levodopa (36).

A reduced absorption rate of Phe by levodopa after oral Phe loads has been demonstrated in Parkinsonism (16-38). The present experiments with loads of Try together with Tyr, Phe and

Gln, respectively, indicate that a general competition may exist between monoamine-monocarboxy acids for intestinal mucosal carriers. This competition may also exist in the transport of Try through the blood-brain barrier and through the neuromembranes in the CNS as indicated by the above cited in *ex vivo* studies.

It may also be expected that the absorption of levodopa is affected by the presence of other amino acids. In favour of this assumption is the observation by Cotzias *et al.* (10) that a high protein intake inhibits the therapeutic effect of levodopa and that L-Try depresses the uptake of levodopa in the brain and pancreas in rats (35).

A diminished absorption of Try during levodopa treatment will, especially in cases with subnormal fasting serum Try values, lead to a decreased influx of Try into the brain. The Try in the brain cells may be further diminished by defective transport through the neuromembranes if the number or activity of the carrier sites is also reduced in these membranes. As a consequence of this, the metabolism of Try in the brain will be diminished, resulting in a decreased synthesis of 5-HT, a neurotransmitter proposed to be involved in emotional responses and mood (6). Consequently changes in mental behaviour may be expected (see below).

Other mechanisms by which the synthesis of 5-HT in the brain is reduced during levodopa administration has been shown. The 5-OH-indole hydroxylase, a limiting enzyme in the synthesis of 5-HT, is depressed by levodopa in the brainstem of rats (20) and also by other catechols (14). A competition for the 5-HTP decarboxylase by catecholic acids, increased during levodopa treatment, has also been suggested as causing a diminished synthesis of 5-HT as the enzyme is common to indolic and catecholic acids. A decreased striatal decarboxylase activity in Parkinsonism has been shown (28). A displacement of 5-HT by DA in the serotonergic neurons in the brain has been demonstrated in rats after large doses of levodopa (2, 4-12). Thus many factors seem to have the effect of reducing 5-HT in the brain during levodopa administration.

In parkinsonian patients a direct *in vivo* demonstration of reduced synthesis and turnover of 5-HT in the brain by levodopa is not possible. However 5-HIAA, the main metabolite of 5-HT, is found to be closely correlated in the CSF with

the turnover of 5-HT in the brain (31). Thus an indirect method of measuring the brain metabolism of Try and 5-HT is available by determining 5-HIAA in CSF. These analyses have revealed subnormal values in untreated parkinsonian patients (15, 17, 21, 22, 23, 39) and still lower values are found during levodopa treatment (39). These findings correlate well with the results of animal experiments with levodopa. They demonstrate an interference of levodopa with 5-HT synthesis and metabolism.

Of special interest is the antagonism between levodopa and Try. As shown in the pioneer investigations of Bernheimer et al. (3), the amine metabolites of these amino acids, DA, NA and 5-HT respectively were decreased in the basal ganglia of parkinsonian patients. It was therefore considered logical to treat the patients with both levodopa and Try. Preliminary experiments by Hornykiewicz, Cotzias and others (ref. 5 p. 64) yielded conflicting results. Further experiments in the combined levodopa and Try treatment by Curzon et al. (ref. 5 p. 112) resulted in a decreased effect of levodopa, whereas Papavasiliou et al. (33) did not observe that Try had any action. Chase (7) treated parkinsonian patients orally with 5-HTP together with a decarboxylase inhibitor (Mk. 485) and reported aggravation of the symptoms (no levodopa treatment). Considering the antagonism between DA and 5-HT an exacerbation of the parkinsonian symptoms could be expected.

A more detailed description and analysis of the status of the patients treated with levodopa and Try, the doses and intervals between the doses might possibly offer an explanation of the different results. However the results will presumably depend on the degree of depletion of DA and 5-HT in the brain, the anatomical localization and extension of the pathological processes, factors which escape *in vivo* analysis.

In case 1, in which the *iv* Try infusions were followed by an "awakening effect" a combined oral treatment with levodopa and Try administered together 3 times daily was tried, but little absorption of Try was found. The real improvement of the patient, mentally and motorically was first seen after the introduction of the alternating levodopa and Try treatment, levodopa during the day and Try during the night. This plan for the treatment seems therefore to be the most ade-

quate way of facilitating synthesis of DA and 5-HT and possibly of building up stores of the amines in the CNS. The balanced levodopa and Try treatment is thus a continuation and realization of the original intentions of Hornykiewicz et al. and Cotzias et al.

In the present and a previous paper (26) the beneficial effect of Try on the mental symptoms has been demonstrated in patients who had severely deteriorated mentally during levodopa treatment. The low 5-HT and Try levels in the blood of the patients indicated a far advanced depletion of Try in the body. Whether the Try treatment had any effect on the neuromuscular symptoms is difficult to evaluate inasmuch as the improvement in mental condition allowed the patients to demonstrate a higher degree of agility and motility than before. The improvement seen, especially in case 1 has therefore apparently given a false impression of an improvement in motility. However Moir (30) has recently shown that *iv* infusion of Try (in dogs) not only increased 5-HIAA in the CSF but—surprisingly—also HVA (homovanillic acid, the main metabolite of DA) and to much higher levels than 5-HIAA. The explanation of this interaction between the metabolism of 5-HT and DA is yet unclear but the experiments seem to reveal evidence of a hitherto unknown interaction between tryptophan and dopamine metabolites. If it can be demonstrated that this is due to an increased turnover of DA and that *iv* Try therapy in parkinsonian patients has a similar effect, a biochemical explanation may be offered for the above mentioned improvement in motility.

The mental side-effects of levodopa treatment reported have been conflicting. In some cases an elevation of mood has been reported in others depression (ref. 5 p. 97). Another disease in which similar mental disturbances are seen in connection with low Try in serum is carcinoidosis with liver metastases (24). In both conditions the disturbances may progress to a stuporous state as in case 1 or into a condition difficult to differentiate from senile dementia as in case 2. In these two cases subnormal fasting serum Try values were found as in the carcinoid patients with liver metastases, complicated by mental symptoms (24). In carcinoidosis the mental symptoms can be alleviated by Try medication (24) and this treatment was also beneficial in the above mentioned

parkinsonian patients (cases 1 and 2) in whom the mental symptoms were aggravated during levodopa treatment. There are thus more features in common in the two diseases in spite of the different aetiologies. In carcinoidosis the low Try in serum is consequence of deprivation of Try from the blood due to the consumption of Try in the tumours, whereas in Parkinsonism the low serum Try appears to be due to malabsorption of Try. In both instances the concomitantly low influx of Try into the brain will result in similar metabolic insufficiencies and in similar symptoms. Depression is a predominating symptom in cases of carcinoidosis with low serum Try values and is also frequently seen in Parkinsonism. It is therefore of interest that low 5-HIAA values in CSF are found in endogenous depression (ref 5 p 64) as well as in Parkinsonism. No values have been available for carcinoid patients with depression.

As a prophylactic measure against mental side effects of levodopa, treatment with a protein-enriched evening meal, some hours after the last levodopa dose, is worthwhile trying. If mental side-effects appear 3-5 g L Try should be added to the meal. Whether protein or amino acid therapy may be of any value in correcting the disturbed amino acid metabolism in the brain in Parkinsonism is an open question. As shown by Gerstenbrand and Gröndig (13), there is in the CSF of parkinsonian patients an increased concentration of glycine, serine, cystine + cysteine and methionine and a very low value of Glu as compared with normal subjects. Glycine was considered as the trigger amino acid, inducing changes in the other amino acids (with the exception of Glu). When levodopa was injected intravenously (100 mg), glycine returned to a normal range while the tyrosine level rose both in CSF and blood. Glu remained at an unchanged low level in the CSF. Glycine was considered as an "inhibitor" amino acid which compensates the DA deficiency and therefore returned to normal when DA increased due to levodopa therapy. Of considerable interest is that blood amino acids were normal in parkinsonian patients. This means that the changes in amino acids observed in the CSF mirror a disturbed amino acid metabolism in the brain. Possibly a disturbed transport of the acids from blood to the brain cells may also be taken into consideration.

As mentioned above, case 2 was treated for

some months with a protein meal supplemented by Glu. Whether Glu had any effect is uncertain.

A question of interest is whether the disturbed Try absorption caused by levodopa treatment becomes irreversible if the treatment is continued for years. Furthermore, will Try or protein supplements administered separately from levodopa improve the absorption? The present investigation does not allow any conclusive answers to these questions owing to the few patients treated. It may be noted, however that in case 2 the Try and protein medication restored the Try absorption to normal. The insufficiency in the Try absorption was thus fully reversible. In case 1 the more severe case, only a slight improvement in the ability to absorb Try was seen after intense i.v. and oral Try treatment. In this case the malabsorption seems to have been irreversible.

The patients with senile dementia showed normal serum Try values after a Try load. They were, however not tested with the Try loads combined with the other aromatic amino acids.

In summary this investigation has shown that Try malabsorption seems to exist in most parkinsonian patients during levodopa treatment, and presumably there is malabsorption also of other amino acids. Mental side-effects of levodopa were found to be correlated to the malabsorption of Try in at least two of the patients in whom Try medication brought about an improvement. In addition, evidence has been presented that Try and protein medication are able to restore the depressed absorption of Try loads during levodopa treatment.

The results of this preliminary investigation have to be verified by similar experiments in a larger group of patients. The experiments are published in the hope that others, having access to a larger number of patients, will repeat the experiments.

As regards the question whether low fasting and low Try load serum Try values in patients displaying mental symptoms are pathognomonic for carcinoidosis, it can be stated that this is not the case.

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BIOCHEMICAL EVALUATION OF LOW DOSE OF UROKINASE IN ACUTE MYOCARDIAL INFARCTION

A Double-blind Study

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Abstract. A low dose of urokinase (UK) has been tested in a double-blind study in the coronary care unit for fibrinolytic response in 14 patients suffering from acute myocardial infarction less than 24 hours old, whereas 14 similar patients received placebo. The initial UK dose was around 74 500 CTA units (50 000 Ploug units) and the sustained dose 37 250 CTA units/h (25 000 Ploug units) for 22 hours. Total dose 890 000 CTA units (600 000 Ploug units). The biochemical response was tested by measure of fibrinogen, fibrinogen-related antigens (FRA), plasminogen, euglobulin clot lysis time (ELT), plasma thrombin time, α_2 -antitrypsin, α_2 -macroglobulin, partial thromboplastin time, platelet count, platelet aggregation, and furthermore of CPK, GOT and LDH in serum, and by ECG recordings. The initial dose produced small but significant enhancement of ELT which was maintained only in a few patients by the sustained dose. Plasminogen dropped to around 75% within 24 hours. The fibrinogen concentrations dropped in 4 of 14 patients receiving UK, but increased in all in the placebo group within the first 24 hours. The statistical treatment showed significant differences between the course of the fibrinogen concentrations from the 72-hour period. The initial dose increased significantly FRA in serum samples without EACA, whereas normal levels were found in the placebo group. The highest values were found after 6 hours. α_2 -antitrypsin and α_2 -macroglobulin patterns appeared identical within the two groups. No influence on enzyme or ECG patterns was seen. It is suggested that the sustained dose is too low

of UK, consequently resulting in high prices, and the contaminating coagulative activity (1-4). Very pure preparations are available, although they may still contain some coagulative activity especially demonstrable in low dosage (5-25). Significant fibrinogenolytic and fibrinolytic activity is obtained as illustrated by shortening of euglobulin clot lysis time (ELT) and drop in plasminogen and fibrinogen concentrations by using an initial dose of around 7 000 CTA units/kg b.wt. given within 10 min, and a maintenance dose of 3 600 CTA units/kg/hour for 10-20 hours (5-7). Prentice et al. (25) have shown that a loading dose of around 10 800 CTA units/kg is necessary for obtaining a distinct increase of fibrinogen-fibrin breakdown products in serum and a prolongation of the thrombin time (TT).

Some authors claim to have found some fibrinolytic activity using many times lower doses (28) in the search for a "minkdose" scheme corresponding to trials with low dose of streptokinase (1A).

The aim of this study was primarily to investigate in a double-blind trial whether a low dose of UK, which according to preliminary experiments *in vivo* might produce some fibrinolytic activity was able to maintain fibrinolytic activity during an infusion period of 22 hours in AMI. The coagulation activity of this dose was examined.

The possible clinical effect was evaluated by repeated measurements of the three serum enzymes CPK, GOT and LDH and by frequent ECG recordings in a coronary care unit (CCU) well equipped with monitoring systems.

Urokinase (UK) has been used increasingly over the last 10 years in different thromboembolic diseases (7, 8, 21-27) especially in acute pulmonary embolism (9, 15-29). Only very few reports deal with UK treatment of acute myocardial infarction (AMI) (19).

Two practical problems have limited the use of UK, the difficulty of obtaining sufficient amounts

Table 1. Clinical data

	UK	Placebo
No. of pts.	14	14
Males	11	10
Females	3	4
Average age (y)	62	64
Mainly anterior infarction	7	10
Mainly posterior infarction	7	4
Previous infarction	4	4
Interval from onset of symptoms to UK/placebo (h)		
< 6	5	3
< 12	12	9
< 24	14	14
Mean interval	6	11

MATERIAL AND METHODS

Clinical material

Twenty-eight patient suffering from AMI of less than 4 hours duration and with no contraindications (see below) for UK infusion were selected for the trial (Table 1).

The patients were only included if the chief assistant of the CCU suggested that the patient would survive the experimental period of at least 1 day (optimal wish 3 days).

When it was decided that patient should be admitted, he was given one of 28 boxes, 14 of which contained ampoules of the active UK preparations and 14 placebo. The two kinds of boxes were arranged at random and no one of the staff knew whether the patients received the active drug or the placebo. The code was kept in the factory and not opened until the trial was finished.

One other patient primarily admitted was excluded owing to death within 6 hours, and consequently no biochemical and clinical evaluation could be obtained.

To patients, one from each group, surviving for 12 hours only are included in the material. All patients were treated in our CCU which is well equipped with continuous monitoring alarm system and special equipment for recording of extra syncope.

Contraindications for admission were recent extra-cardiac trauma, active anticoagulant treatment, surgery within the last 3 days, and age above 80 years.

Urokinase dose The initial UK dose was 50 000 Ploug units (around 74 500 CTA units) given within 10 min and the continuous dose 25 000 Ploug units (around 37 250 CTA units)/hour for 22 hours. The total dose was 600 000 Ploug units (around 890 000 CTA units). The UK was given intravenously dissolved in 5% glucose and so was the placebo.

The patients received no other active antithrombotic treatment. All complications were treated according to the usual principles of the CCU.

Diagnosis, laboratory and clinical control

The diagnosis was in all cases based on typical history on distinct enzymic changes with elevation of CPK, GOT and LDH, and on characteristic ECG patterns.

Fibrinolytic activity Blood samples were drawn before infusion and 6, 12, 18, 24, 36, 48 and 72 hours after the beginning of infusion and were tested for fibrinogen, fibrinogen-related antigens (F.R.A.) (from samples both with and without ϵ -aminocaproic acid (\pm EACA), plasminogen, α_2 -antitrypsin and α_2 -macroglobulin and, always on freshly withdrawn blood, for ELT plasma TT and platelet aggregation.

In 8 patients receiving UK and in 6 receiving placebo, blood was also drawn 10 min after infusion of the initial dose and estimated for ELT TT and F.R.A. \pm EACA.

Fibrinogen was quantitated immunologically by a modification of the microrocket method of Laurell (22). Plasminogen (casein method), ELT TT F.R.A. (TRICH), α_2 -antitrypsin and α_2 -macroglobulin (radial immunodiffusion) were estimated as described elsewhere (11, 12, 18, 20).

The results of the measurements of fibrinogen, plasminogen, α_2 -antitrypsin and α_2 -macroglobulin obtained in the first 72 hours were evaluated statistically by fitting an orthogonal second degree polynomial to the individual concentration/time curves (7 values in each patient). By using orthogonal parabolas the three parameters, the mean, the linear and the quadratic components, were accordingly estimated independently of each other. The statistical significance was tested by Student's *t*-test and the statistical significance obtained for the quadratic component of fibrinogen and plasminogen was furthermore checked by Wilcoxon's non-parametric test. All calculation was performed by cand. polyt. H. Engher-Pedersen.

Plasma from 6 patients from each group (left from + EACA 10^{-4} mol/l) were estimated by agar immunoelectrophoresis ad modum Scheidegger (26) against anti-fibrinogen.

Platelet aggregation was measured in a Born aggregometer employing freshly drained platelet-rich citrated plasma at 37°C under continuous stirring. Aggregation was induced by ADP final concentration 0.5 mmol/l.

Nineteen and 20 measurements, respectively were carried out in 8 patients receiving UK and in 11 receiving placebo in 4 and 3 of these, respectively measurements were performed both before, during and after infusion. In 3 patients measurements were carried out before intracaine administration. In all other patients Elocaine was given once or less continuously during the experimental period and most of the patients also received one or more drugs such as digoxin, spinaline, furosemide, pethidine etc., but not diazepam or drugs containing acetylsalicylic acid known to have significant influence on platelet aggregation.

Coagulation studies. PTI (partial thromboplastin time) was estimated in vitro by means of the kaolin-certhale-clothing time (Behringwerke Marburg/Labo). Freshly drawn platelet-free citrated plasma was incubated in UK in concentrations of 1.5–1500 CTA units/ml diluted in buffered saline. The UK concentrations exceed those obtained in vivo. Normal values from 40–55 sec.

Enzymes. Blood was collected for examination for CPK, GOT and LDH at the same periods as for fibrinolytic studies. The enzymic changes were evaluated by fitting a parabola through 3 consecutive sample results, with the

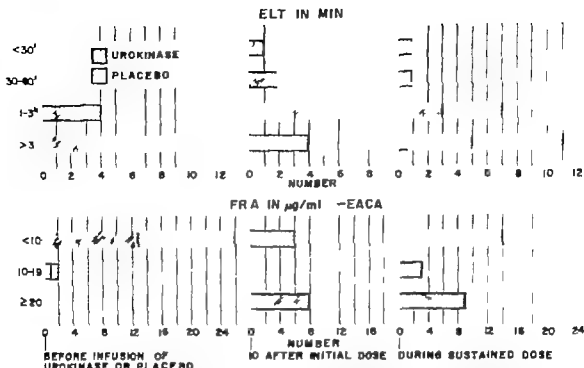


Fig. 1 Upper section: ELT before and 10 min after infusion of UK or placebo in 8 and 6 patients, respectively and in 10 and 10 during sustained infusion, which might be after 6, 12 or 18 hours. Lower section: F.R.A.

values (without EACA) from the patients before infusion of UK or placebo, from 8 and 6 patients, respectively 10 min after infusion, and from all patients 18 hours after infusion.

patients maximum sample within the first 48 hours in the middle. By interpolation the maximal value and the time for obtaining the maximum value were found. Furthermore the slope of the descending part of the parabola was evaluated (drop in units of the enzyme per 6 hours after maximum) and the duration of enzymic values higher than the pretreatment values was estimated. Wilcoxon's rank-sum test was used for comparison between the evaluated parameters in the two groups.

Routine laboratory examinations: BP, ESR, Hb, leucocytes, thrombocytes, serum creatinine, serum cholesterol, lipoproteins, blood sugar, serum uric acid, PPV, uric acid, etc. were estimated. These examinations, like the determination of enzymes and fibrolytics, were performed by the Department of Clinical Chemistry.

Arrhythmias: The CCU is equipped with special arrhythmia monitors for each patient. These monitors were adapted to count all ectopics during the first 48 hours with ECG documentation of every arrhythmia occurring.

RESULTS

Fibrinolytic activity

10-min samples. ELT and F.R.A. (without EACA) values obtained before and 10 min after infusion of initial dose of UK or placebo (Fig. 1) from 8 and 6 patients, respectively are illustrated

ELT was identical in both groups before injection. Significant shortening of ELT developed after UK in the 8 patients, in one to below 30 min, whereas unchanged ELT values were seen in the 6 patients in the placebo group.

F.R.A. values were at zero time (immediately before infusion of UK or placebo) increased in one patient from each group in serum samples with and without EACA, presumably due to spontaneous fibrinolysis. Otherwise F.R.A. values were below 10 µg/ml, normal in serum samples with EACA during the total experimental period of 72 hours, also after infusion of UK. F.R.A. values in serum samples without EACA were below 10 µg/ml at zero time in the other 26 patients, significantly increased in the 10-min samples in the 8 patients receiving UK, and remained unchanged after placebo infusion.

No prolongation of TT was seen in any of the 10-min samples.

Sustained fibrinolytic activity The ELT values found in 10 patients tested once during the sustained infusion of urokinase (Fig. 1 upper section right-hand part) showed only slightly in-

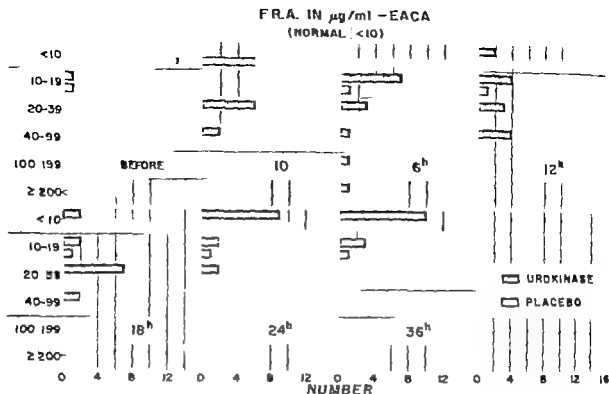


Fig. F.R.A. values (without EACA) from all patients before, during and after UK or placebo.

creased activity compared to those obtained in 10 patients tested during infusion of placebo.

The values of F.R.A. in the 18-hour samples (Fig. 1 bottom section, right hand part) were in the UK group significantly increased in serum samples without EACA except in 2 patients who maintained a level of F.R.A. between 10 and $9 \mu\text{g}$ during the whole 72-hour observation period. The values found at the different times within the first 36 hours are shown in Fig. 2. In general the highest values—and the widest range—were found after 6 hours, whereas the 36-hour

samples are very much like those found in the placebo group.

Fibrinogen (Table II). Mean concentration before infusion of UK or placebo (14 patients in each group) was 3.62 and 3.66 g/l, respectively and after 24 hours 3.88 and 4.37 (13 in each group) with average increase of 0.26 g/l and 0.71 g/l. The difference is not significant ($p \sim 0.05$) evaluated by Wilcoxon's non parametric test. It might be added that no increase in fibrinogen was seen in 4 of the patients in the UK group. One in this group showed identical results at zero time and after 24 hours, whereas a distinct rise was recorded in all 13 patients in the placebo group surviving for 24 hours or more. However the statistical evaluation of the fibrinogen concentrations obtained during the whole 72-hour period using orthogonal parabolas showed a significant difference for the quadratic component ($p < 0.01$), as also confirmed by Wilcoxon's non-parametric test (Table III). This is possibly due to 1 or 2 lower mean values in the UK group at some interval within the 72-hour period.

Agar immunoelectrophoresis. The patterns ob-

Table II. Fibrinogen and plasminogen before and 24 hours after infusion

	Fibrinogen (g/l)		Plasminogen (%)	
	UK	Placebo	UK	Placebo
Mean before	3.62	3.66	103	102
Mean after 24 h	3.88	4.37	76	99
Average increase	0.26	0.71	-27	-3
Increase/decrease	9/13	13/13	13/13	1/13

$p \sim 0.05$, $p < 0.001$.

Table III. Statistical evaluation of differences between some parameters in UK and placebo groups

All differences tested by Student's *t*-test

	Components of orthogonal parabolas		
	Mean	Linear	Quadratic
Fibrinogen	NS	NS	$p < 0.01^a$
Plasminogen	$p < 0.001$	NS	$p < 0.05$
α_1 -antitrypsin	NS	NS	NS
α_2 -macroglobulin	NS	NS	NS

NS = non-significant.

Also significant ($p < 0.01$) when tested with Wilcoxon's non-parametric test.

tained were inconclusive as in some patients they were abnormal at admission before infusion.

Plasminogen. A distinct decrease in plasminogen concentration was seen in the UK group after 24 hours, with a mean value of 76% whereas no change appeared in the placebo group (99% mean value after 24 hours). All patients in the UK group showed a decreased concentration (Table II). (Plasminogen for the whole 72 hour period, see Table III)

Differences between concentrations of α_1 -antitrypsin in the two groups could not be demonstrated (Table III). The daily increase was 10% and 13% respectively.

No significant differences were seen between concentrations of α_2 -macroglobulin within the 72-hour period in the two groups using orthogonal parabolas. If however concentrations before infusion of UK or placebo were compared with values obtained after 6 and 12 hours, the concentration was lower in the UK group than in the placebo group, but the difference was not significant (Table II).

Thrombin times during sustained infusion were identical in the two groups, being normal in both groups.

Platelet aggregation

The patterns found before infusion of UK or placebo were very different (Fig. 3). In 1 patient the curve was normal, in 3 the primary aggregation was very pronounced with nearly maximal decrease in optical density. Several patients showed a reduced primary aggregation, which in some was followed by a quick disaggregation. It is

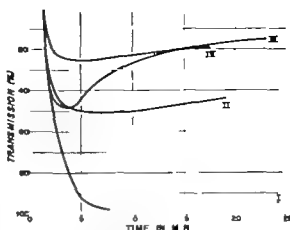


Fig. 3. Platelet aggregation after addition of ADP to citrated platelet-rich plasma (final concentration 0.5 mmol/l) at 37°C. The results shown are from blood samples taken before the infusion period. Platelet counts are in all cases within the normal range. Four types of aggregation patterns found in the present material are illustrated. I = patients with increased aggregation, II = normal aggregation, III = decrease of primary aggregation and disaggregation, and IV = decrease of primary aggregation.

unknown which drugs these patients might have taken at home before admission to hospital. The curves found during the infusion period and the following days were as different from case to case as those already described, but rather consistent in the same patient. Neither urokinase nor lidocaine was significant for the patterns obtained.

Clotability

No clotting activity was demonstrated in vitro by PTT with concentrations from 1.5–1 500 CTA units/ml plasma (Table IV).

Table IV. Influence of urokinase *in vitro* on PTT (sec)

Plasma samples with and without EACA (final concentration 10^{-3} mol/l) incubated for 1 min with UK at 37°C before clotting

UK (CTA/ml plasma)	+ EACA	- EACA
0	38	37
1.5	41	41
15	41	38
37.5	40	40
75	39	39
150	40	38
300	37	38
600	38	39
750	39	39
1 500	38	38

Table V Clinical results

	UK	Placebo
No. of patients	14	14
Survivors	12	11
Early deaths (<24 h)		
Cardiac insufficiency	1	
Rupture		1
Later deaths (>24 h, <4 weeks)		
Cardiac insufficiency	1	1
Pulmonary embolism		1
Total	2	3

Enzymes

The maximal values of CPK and GOT the time for obtaining the maximal values (recorded from the time when the pretreatment sample was taken) and the drop in the enzymic values during the first 6 hours after maximum showed no significant differences between the two groups when tested by the Wilcoxon's rank sum test. Values below the pretreatment values were not obtained in half of the cases in either group within the observation period. The run of the LDH curves varied individually so much in both groups that statistical evaluation served no end.

Clinical results

Twelve survived in the UK group and 11 in the placebo group (Table V). There was one early death in each group. Differences in ECG patterns were not demonstrable as regards ST elevations, frequency or types of arrhythmia.

DISCUSSION

The dosage scheme generally recommended for urokinase in thrombolytic therapy is around 7 200–10 800 CTA units/kg as initial dose, given within 10 min, and a maintenance dose of around 3 200 CTA units/kg/hour which is around 630 000–800 000 CTA units initially and around 250 000/hour. This dose produces a significant increase in fibrinolytic and fibrinogenolytic activity in the circulating blood.

Suyama and Shibuya (28), however, claim that even a dose as small as 5 000 Ploug units, corresponding to around 7 450 CTA units given within 10 min accelerates whole blood clot lysis and ELT and prolongs the plasma thrombin time

significantly and that daily injections of 7 450–44 700 CTA units are followed by angiographically proven thrombolysis. The Japanese may possibly be more sensitive to urokinase.

Our loading dose of around 74 500 CTA units and continuous dose of around 37 250 CTA units were chosen as a less expensive compromise between the high and the low doses.

The significant drop in plasminogen in all UK patients proved that the UK doses given had been active but did not necessarily result in fibrinolysis or fibrinolytic. Fibrinogen concentrations generally rise during the first 24 hours in patients suffering from AMI (10–12), and generally by 0.5–1.00 g/l/day. In this study with partly selected patients 4 in the UK group had no increase within the first 24 hours and 1 maintained the level found before infusion of UK, whereas all patients receiving placebo infusion had an increase of fibrinogen concentration during the first 24 hours. By using orthogonal parabolas for evaluation of the whole 72-hour period, it was shown by the quadratic component that significantly lower fibrinogen concentrations were present in the UK group at some period during the 72 hours, possibly due to the fact that the fibrinogen concentration dropped in some patients in the UK group whereas rather high fibrinogen concentrations were found in other patients late during the 72-hour period within the same group. Immunological methods for the measurement of fibrinogen concentrations might not be the best for the present purpose due to the fact that some fibrinogen breakdown products are nearly as active in this method as fibrinogen itself. Distinct *in vivo* fibrinogenolysis and/or fibrinolysis should, however, result in an increase of F.R.A. as measured by TRCHII (+BACA in serum), although most of the higher molecular weight fibrinogen breakdown products become incorporated within the clot formed *in vitro* by this method.

The drop in the α_2 -macroglobulin normally seen during infusion of higher doses of UK (2) and during conventional streptokinase therapy (6, 12, 23) was not seen in the present study although the statistical treatment indicated that the concentration was lower during the infusion of UK. This difference was not statistically significant. The sensitivity of the radial immunodiffusion is not the best at high concentrations of α_2 -macroglobulin and it is hardly possible to distinguish a

small decrease, if present, by this method, and especially not as the concentrations of α_2 -macroglobulin were high in the patients concerned (120% and 140% respectively).

The biochemical data appear to show that, whereas the initial dose did increase the activator activity as illustrated by shortening of ELT and by increase of F.R.A. in serum samples without EACA in vitro, such activities were maintained only in a few by the sustained dose. It is possible that doubling of this dose might result in maintenance of an increased activator level.

The inhibitory effect of F.R.A. and different drugs on platelet aggregation is well known (3 13 16). An increased extent of aggregation has been described in patients with AMI (30).

Our results obtained with the Born aggregometer were extremely varying and did not allow any final conclusion. Neither F.R.A. nor UK exerted any influence on the aggregation parameters. A more detailed study of aggregation parameters in AMI and of the influence of various complications, such as shock and of different drugs, might be worthwhile.

No influence on enzyme and ECG patterns as described during streptokinase therapy (17 24) was observed.

ACKNOWLEDGEMENTS

This study has been supported by grants from the Danish Hospital Foundation for Medical Research, Region of Copenhagen, Faroe Islands and Greenland, and from the Statens Lægemiddelkontrollen Forskningsråd.

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INTRAATRIAL AND ATRIOVENTRICULAR CONDUCTION DISTURBANCES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract. In a prospective study of 171 patients with confirmed acute myocardial infarction (AMI) intraatrial disturbances of conduction have been demonstrated in 20. The mortality among these patients was 10%. Six patients later developed atrioventricular (AV) conduction disturbances, of considerable severity in five. First degree AV block was recorded in 11 patients, 5 of whom showed subsequent progression to higher degrees of AV block. Second degree AV block was observed in 16 patients, with 31% mortality. In 7 of the patients progression to third degree AV block as subsequently observed. There was no connection between the site of the infarction and the mortality or progression to higher degrees of AV block. Third degree AV block was recorded in 21 patients. Of 12 with infarctions of the anterior wall 5 died. Of 9 with infarctions of the posterior wall 2 died. In 21 patients with third degree AV block regression to sinus rhythm occurred in 16.

The introduction of continuous ECG monitoring in intensive coronary care units (CCU) has revealed a high frequency and early occurrence of dysrhythmias and conduction disturbances during the course of acute myocardial infarction (AMI) (5, 7, 12, 18, 19).

Disturbances of the cardiac conduction system in AMI are usually transient and are reported to occur in 5-15% of cases (1, 11, 12, 19). It is well substantiated that the mortality increases with the degree of conduction inhibition, which is more often due to ischemia, haemorrhage or oedema with secondary lymphocytic infiltration in the conduction system than to anatomical interruption (1, 3, 13, 14, 17).

In the study to be reported we investigated the occurrence, progression and regression of intraatrial and atrioventricular (AV) conduction disturbances in patients with AMI with a particular view to relations to mortality and site of infarct judging by the ECG tracings.

MATERIAL AND METHOD

During the period Nov. 24, 1967 to Dec. 31, 1968, total of 171 patients with confirmed AMI are admitted to the CCU of the Copenhagen Municipal Hospital (2).

The clinical condition on admission is classified as "mild" in the absence of decompensation or arterial hypotension, as "severe" in the presence of decompensation or arterial hypotension, and as "cardiogenic shock" if there were typical signs of acute heart pump failure.

During the monitoring period the ECGs for all patients were continuously transferred to magnetic tapes and analysed daily for disturbances of rhythm and conduction.

The present material comprises 60 patients in whom intraatrial and atrioventricular conduction disturbances were observed. Patients with conduction disturbances that might be drug-induced were not included. The interpretation of transient changes in the P wave, in the form of diphasic, split, or negative waves, is uncertain, and such cases were omitted from the study.

Intraatrial and AV conduction disturbances occurring during the last minutes before death in patients who were in cardiogenic shock are interpreted as terminal and were omitted from the study as were also transient conduction disturbances immediately after DC defibrillation.

Definitions

Sinoatrial block (type 1). Delayed occurrence of the P QRS-T complex with a pause in the basic rhythm of less than twice or almost twice the normal duration of the P-P.

Sinus arrest (sinoatrial block type 2). Drop-out of sinus beats with pauses in the basic rhythm which is twice the duration of the normal P-P interval or more.

Intraatrial dissociation or atrial parasystole. Occurrence of two P waves, with no time relation to each other, one bearing constant relation to the QRS complex.

First degree AV block (1° AV block). Sinus rhythm, 1:1 AV conduction, but prolonged PQ interval > 0.22 sec.

Second degree AV block (2° AV block). Wickeback periods or 2:1, 3:1, 3:2, etc. AV conduction with constant PQ interval—occurring constantly or periodically.

Third degree AV block (3° AV block). Atrial de-

Table I Type of intraatrial conduction disturbance and mortality related to site of infarction in 20 patients with AMI

	Site of infarction			Total	Mortality
	Anterior wall	Posterior wall	Indefinite		
Stems arrest	1	4	1	6	1
Sinoatrial block	1	6	0	7	0
Wandering atrial pacemaker	0	4	0	4	1
Intraatrial dissociation	1	2	0	3	0
Total	3	16	1	20	2
Total for 171 pairs with AMI	81	66	24	171	30 (18%)

polarization with P waves and total AV dissociation with idioventricular or junctional rhythm.

Therapeutic principles

The therapeutic principles were correction of cardiac decompensation, arterial hypotension, electrolyte disturbances, and changes in the acid-base balance.

Treatment of intraatrial conduction disturbances was instituted on recording more than 3% sinoatrial block and/or stems arrest. It consisted of 1-4 injections of atropine, 0.5-1 mg, 3 or 4 times daily.

1 and 2° AV block were treated only in the presence of bradycardia and/or hypotension. In such cases atropine was administered as described above. If atropine was ineffective, it was supplemented by infusion of isoprenaline 5 mg in 1000 ml isotonic glucose, 10-30 drops/min. To counteract any ectopic rhythms a lignocaine drip (1000 mg in 1000 ml isotonic glucose) was kept in readiness. In a few cases, moreover, treatment with glucocorticoid (Soliasol), 140 mg i.v. 4 times daily was attempted.

Every case of 3° AV block was primarily treated with atropine, isoprenaline, and possibly glucocorticoid as mentioned above. If the patients had Stokes-Adams attacks or if, despite the medication, they continued having bradycardia with reduced cerebral or cutaneous circulation and hypotension, or if they had repeated ventricular tachyarrhythmias, a transvenous pacemaker catheter was inserted into the right ventricle under fluoroscopic control and connected to an Electronic Demand pacemaker.

RESULTS

Intraatrial conduction disturbances

Intraatrial conduction disturbances were recorded in 20 patients (12%) during the observation. Sixteen of these had posterior wall infarction (Table I).

Sinoatrial block (type 1) was seen in 7 patients. One of these developed later transient 3° AV

block and one developed AV dissociation with nodal escape rhythm. None in this group died.

Repeated sinus arrest or sinoatrial block (type 2) was seen in 6 cases. The period of asystolia ranged from 1 to 4 sec. One patient developed later transient 1° AV block and 3 developed 3° AV block; one of the latter died.

One of 4 patients with wandering atrial pacemaker died. He had a posterior wall infarct and was in cardiogenic shock on admission.

1° AV block

In 18 patients (11%) 1° AV block was recorded, in all but one of them arising during the observation period. Table II presents the progression and regression in relation to the site of the infarct. Three patients died, one following progression into 2° AV block, one following progression to 3° AV block, whereas one developed ventricular fibrillation and died in intractable asystole after DC defibrillation.

2° AV block

In 16 patients (9%) 2° AV block was observed, occurring in all cases during the observation—in 10 cases from sinus rhythm, in 3 from atrial fibrillation, and in 3 from 1° AV block.

The relation to the site of infarct is shown in Table III, which also shows that almost half of the patients returned to sinus rhythm or atrial fibrillation, whereas the other half progressed to 3° AV block.

One patient had 2° AV block of Wenckebach type and regressed to sinus rhythm.

Two patients, both with posterior wall infarct, had Mobitz block type 2. Both developed 3° AV block. One of them died, the other regressed to sinus rhythm.

The remaining 13 patients had 2:1 or 1:1 AV block. Four of these died. Two of the deaths occurred after progression to 3° AV block, both were in cardiogenic shock on admission. One died from rupture of the myocardial wall after regression to 1° AV block, and one in a state of intractable cardiogenic shock without progression from 2° AV block.

3° AV block

In 21 patients (12%) 3° AV block was recorded, in 5 of whom it was present on admission.

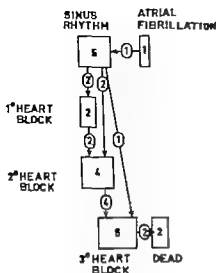


Fig. 1 Progression to 3 AV block developing during monitoring in 5 patients with posterior wall myocardial infarction.

Of these 21 patients 9 had posterior wall infarct and 12 anterior wall infarct. In all cases of anterior wall infarct and in 2 of those with posterior wall infarct there were also widened QRS complexes.

Table IV lists the times of recording 3 AV block in the 21 patients in relation to the presumed time of onset of the infarction.

From Table V it can be seen that 16 patients regressed to sinus rhythm. Two patients died after having attained sinus rhythm, one of them after the monitoring had been discontinued, so that the terminal rhythm is unknown. The other patient died in intractable cardiogenic shock. Five pa-

Table II. Outcome in 18 patients with AMI complicated by 1 AV block

	Sets of infarction			No. of deaths
	Anterior wall	Posterior wall	Indefinite	
Regressed to sinus rhythm	5	4	11	0
Remained in 1 AV block	0	1	2	1
Progressed to 2 AV block	1	2	3	1
Progressed to 3 AV block	2	0	2	1
Total	8	7	18	3

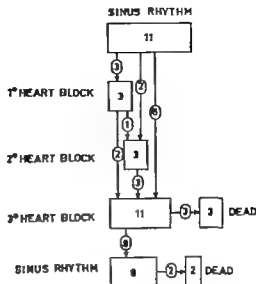


Fig. 2 Progression to 3 AV block developing during monitoring in 11 patients with anterior wall infarction.

tients died in 3 AV block. In one of the two patients who were treated by transvenous intracardiac pacing the direct cause of death was pulmonary embolism and in the other autopsy showed the right ventricle to be filled with thrombotic masses. In three drug-treated patients the cause of death was intractable asystole following DC defibrillation of ventricular fibrillation in two, rupture of the anterior wall of the left ventricle in the third.

Fig. 1 shows the development into 3 AV block in 5 patients with posterior wall infarct in whom the block occurred after admission. Only one patient showed direct progression from sinus rhythm into 3 AV block.

Fig. 2 presents the development into 3 AV block in 11 patients with anterior wall infarction, all of whom developed the block after admission. In 6 of these patients the 3 AV block developed suddenly direct from sinus rhythm.

Table VI shows the mortality in relation to the degree of conduction inhibition and Table VII the occurrence of the various degrees of heart block as well as the mortality in relation to the localization of the infarct, judging by the ECG

DISCUSSION

During the course of AMI attention should be directed to disturbances in the impulse formation and impulse conduction which involve a risk of

Table III. Outcome in 16 patients with AMI complicated by 2° AV block

	Site of infarction		Total	No. of deaths
	Anterior wall	Posterior wall		
Regressed to trial fibrillation or sinus rhythm	3	4	7	0
Regressed to 1° AV block	1	0	1	1
Remained in 2° AV block	1	0	1	1
Progressed to 3° AV block	3	4	7	3
Total	8	8	16	5

developing into fatal dysrhythmias, conduction disturbances, or heart pump failure.

Intraatrial conduction disturbances appear to have been little heeded although they occur in 10–12% of all patients with AMI. Such conduction disturbances are common in occlusion of the right coronary artery but may also be observed in occlusion of the circumflex branch of the left coronary artery. Thus it is rather the localization than the extent of the infarct which decides the occurrence of a conduction disturbance (20). Although the total mortality for these patients proved to be low about 10% (cf. Table I), subsequent AV conduction inhibition occurred in 6 of the 20 patients in the present study severe in 3.

The incidence of 1° AV block (11%) in patients with AMI in the present series corresponds to the findings of others (1, 4, 10, 16). As is apparent from Table II, 11 of 18 patients regressed to sinus rhythm, whereas 5 progressed to higher degrees of AV block, corresponding to the

Table IV. Time of onset of 3° AV block in relation to the onset of AMI in 21 patients

Site of AMI	Hours between onset of symptoms and development of 3° AV block					Total
	1-4	25-48	49-72	>72		
Anterior wall	2	6	4	0		12
Posterior wall	4	2	1	2		9
Total	6	8	5	2		21

Table V. Outcome in 21 patients with AMI complicated by 3° AV block

	Site of infarction		Total	No. of deaths
	Anterior wall	Posterior wall		
Regressed to sinus rhythm	9	7	16	2
Remained in 3° AV block	3	2	5	5
Total	12	9	21	7

studies of Lown (15) and Brown et al. (1), in which about 25% of the patients with AMI and 1° AV block progressed to higher degrees of AV block.

Although all investigations of this nature are based on small series, the incidence and mortality among the present patients with AMI of 2° AV block seems to correspond very well to the findings of others (1, 4, 10, 16). There was no relationship between the localization of the myocardial infarct and the tendency to progression to 3° AV block. Moreover the localization of the infarct appeared to have no influence upon the mortality among these patients. Therefore it is presumably the extent rather than the site of the infarct which conditions the occurrence of the conduction inhibition and the increased mortality within this group.

When AMI was complicated by 3° AV block, the present study like others previously showed a considerable increase in mortality (1, 9, 12, 19).

In the present series the mortality was particularly high if the 3° AV block occurred in pa-

Table VI. Degree of AV block in relation to clinical condition on admission and outcome in 55 patients with AMI

N = no. of patients, n = no. of deaths

AV block	Clinical condition						
	Mild		Severe		Shock		Total
	N	n	N	n	N	n	N
1	7	1	10	2	1	0	18
2°	5	1	8	1	3	3	16
3	4	2	12	1	5	4	21
Total	16	4	30	4	9	7	55

Table VII. Site of infarction in relation to degree of AV block and outcome in 35 patients with AMI

N = no. of patients; D = no. of deaths

Site of infarction	AV block					
	1		2*		3	
	N	D	N	D	N	D
Anterior wall	8	1	8	3	12	5
Posterior wall	7	1	8	2	9	2
Indefinite	3	1	0	0	0	0
Total	18	3	16	5	21	7

patients with anterior wall infarction, also in accordance with previous findings (1 9 12, 19).

Sutton and Davies (21) in microdissection of the conduction system in patients with 3 AV block and anterior wall infarct, have demonstrated injury to the right bundle branch and the two left bundle branches of the conduction system. This explains the general observation that in anterior wall infarction 3 AV block is accompanied by idioventricular rhythm with widened QRS complexes.

In anterior wall infarction the infarcted area must necessarily be of large extent, owing to the scattered anatomical placement of the conduction system, before a 3 AV block can arise. Thus the extent of the infarct is responsible for the interruption in conduction between atria and ventricles and for the increased mortality among these patients. Furthermore, the progression to 3 AV block often occurs abruptly direct from sinus rhythm, activating ectopic foci in the branches of the conduction system, or ventricular myocardium with slow ventricular rate, a tendency to Stokes-Adams attacks, repeated ventricular ectopias or entricular tachyarrhythmias. All this further aggravates the prognosis if corrective measures are not instituted in time (1 9 12, 18).

When posterior wall infarcts are complicated by 3 AV block, the infarcts are usually of fairly small size. On microdissection they have been seen to comprise, *inter alia*, the area around the bundle of His, secondary to occlusion of the right coronary artery proximal to the departure of the AV nodal branch. This explains the common occurrence of idioventricular rhythm at the same time (8). In the present study as well as in previous ones, there has been in practically all cases a

gradual progression from low to higher degrees of conduction inhibition in cases with posterior wall infarction (1 11 12, 18).

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THE PROGNOSIS OF PATIENTS WITH ACUTE MYOCARDIAL INFARCTION TREATED WITH TRANSVENOUS ELECTRICAL PACING OF THE HEART

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Abstract. Thirty-one (7%) of 404 patients with acute myocardial infarction (450 consecutive admissions) had temporary transvenous pacemaker electrode inserted and were artificially paced. The indications for electrical stimulation of the heart were complete heart block in 28 patients, AV block II in two, and extreme sinus bradycardia and nodal bradycardia in one patient. The 31 paced patients are followed up for 2 years or more (mean follow-up time 33 months). Twelve (39%) died during their stay in hospital and another 10 (32%) during the follow-up period. In 21 of these 22 patients the cause of death could be determined; 11 died suddenly. The cumulative survival rate after 25-36 months for the non-paced patients was 53% and for the paced patients 25% (statistically significant difference ($p < 0.01$)). Age differences between the paced and the non-paced patients were small and could not explain the differences. The prognosis for the temporarily paced patients in this report was poor. Even if most of them died suddenly it is doubtful whether the prognosis could be significantly improved by long-term cardiac pacing, as the myocardial damage was found to be extensive in the autopsied cases.

Complete heart block complicating acute myocardial infarction (AMI) occurs most commonly in infarction of the inferior wall of the heart. It is said to be due to inflammation and oedema, involving the area around the AV node (7), which follow the occlusion of the right coronary artery or of a dominant circumflex branch of the left coronary artery. Mortality from this type of complete heart block has been reported to be only slightly higher than that from inferior infarction not complicated by block* (4).

Complete heart block complicating infarction of the anterior wall of the heart is due to damage to the two main branches of the conduction system. The injury is caused by the occlusion of the descending anterior branch of the left coronary artery and this results in necrosis of the

muscles of the anterior part of the heart and atrial septum (27). This type of complete heart block has been reported often to be preceded by unilateral bundle branch block, primarily on the left side, or bilateral bundle branch block (BBBB) and/or AV block II of Mobitz' type II, unless it occurs acutely without previous signs of damage to the conduction system (13). Even if electrical pacing of the heart results in establishment of an adequate heart rate in complete heart block complicating infarction of the anterior wall of the heart, the patients often die of cardiac shock (18).

During the period Jan. 1 1968-Dec. 31 1969 31 patients with AMI were treated in the Coronary Care Unit (CCU) at the Department of Medicine, Serafimerlasarettet, Stockholm, with temporary transvenous electrical pacing of the heart, the indications being complete heart block, extreme nodal bradycardia or sudden cardiac arrest. This paper reports the survival rate of these patients during their stay in hospital and the overall survival rate during the first three years after their discharge.

METHOD

The temporary transvenous pacemaker electrode is inserted in the Catheterization Laboratory adjacent to the CCU in the first insertion: unipolar flexible electrode (Elema EMT 388) was used, inserted through the external jugular vein into the right ventricle. Since March 1969 slightly stiffer, bipolar pacemaker electrode (USCI 3652) has been employed, introduced via the left internal carotid vein.

The threshold for effective myocardial stimulation is tested twice daily and pacing is initiated at V above the stimulation threshold. On failure of pacing the out-

Table I. Overall survival rate in the paced patients

Follow-up time (mo.)	Alive at the beginning of the interval	Deaths during the interval	Withdrawals during the interval	Overall survival rate (%)
0-3	31	12	0	61.3
4-6	19	4	0	48.4
7-12	15	1	0	43.2
13-24	14	3	1	35.2
25-36	10	2	6	25.1

put from the right ventricle is recorded, i.e. ventricular electrogram is taken by means of the pacemaker electrode. The output from the right ventricle should be at least 3 mV to avoid deficient triggering. An external QRS-inhibited generator (Devices, Elema prototype or Medtronic) is used for pacing. The electrode has not been removed until at least 48 hours of AV conduction has been registered.

In order to obtain an adequate trigger mechanism the bipolar electrode is modified in the following way: the indifferent pole of the electrode is isolated and a needle is inserted subcutaneously below the left costal margin and is used as anode, thereby converting the bipolar electrode into a unipolar one.

CASE MATERIAL

This series included 404 patients with AMI who on 450 occasions were treated in the CCU. 31 of these patients had an endocardial pacemaker electrode inserted. Eighteen were men and 13 women, their ages ranged from 46 to 81 years (mean 64.1) and from 57 to 86 years (mean 71.4), respectively. The overall mean age (404 patients) was 63 years for the male patients and 71 years for the female patients.

The criteria on which the admission of these patients was based, the treatment programme and the CCU were described in a previous paper (24). Details of the histories and treatment of these 404 patients were reported by Asplund et al. (1). The indication for pacing in the 31 paced patients was complete heart block in 28, AV block II associated with slow heart rate in two, and extreme nodal bradycardia and hypotension in one pa-

tient. Thus 7% of the patients in this series were treated with electrical pacing of the heart. In one case external transthoracic electrical pacing was applied before using endocardial electrical stimulation, as reported in a previous paper (9).

RESULTS

Heart rhythm before electrical pacing

Ten patients changed from sinus rhythm, which in some cases was associated with AV block I, to complete heart block, 5 had complete heart block on admission, 6 suddenly developed asystole followed by complete heart block. Four patients had AV block I changing to AV block II and later to complete heart block and 3 developed AV block II changing to complete heart block. Of the 3 patients without registration of complete heart block, two had AV block II with bradycardia and one nodal bradycardia with hypotension. In two cases Wenckebach's periodicity preceded and in one case it followed complete heart block.

Bundle branch block

Out of the 26 patients who were found to have some type of conduction disturbance before they developed complete heart block, 4 had left anterolateral bundle branch block (LALBBB), BBBB, 1 RBBB and 1 LBBB. In the case in which BBBB preceded complete heart block the patient was treated with long-term electrical pacing because complete heart block was found to persist 1 month after pacemaker therapy had been instituted. This patient had extensive infarction of the anterior wall of the heart but survived and was discharged from hospital. However 9 months after the appearance of complete heart block she suddenly died. The other patient with BBBB died 19 months after the occurrence of complete heart block.

Hypotension, cerebral involvement and type of treatment given before electrical pacing of the heart

Four patients developed hypotension before the appearance of a conduction disturbance. In 3 cases the bradyarrhythmia was associated with cerebral confusion. One patient was given digitalis and two received lignocaine immediately before they developed bradyarrhythmia.

Table II. Overall survival rate in the non-paced patients

Follow-up time (mo.)	Alive at the beginning of the interval	Deaths during the interval	Withdrawals during the interval	Overall survival rate (%)
0-3	373	104	4	72.0
4-6	265	14	0	68.2
7-12	251	21	0	62.5
13-24	230	24	75	54.7
25-36	129	3	99	52.6

AV conduction during cardiac pacing

In 22 cases the AV conduction returned during cardiac pacing. The patients were paced for 1-20 days (mean 7.0). The duration of AV conduction disturbance during the pacemaker treatment was 0-10 days (mean 4.0).

Overall survival rate

Out of the 31 patients 1 died during their hospitalization and 19 (61%) survived and were discharged from hospital in satisfactory condition.

At the end of one year after the occurrence of myocardial infarction 14 patients (45%) were alive. After a follow-up time of two years or more, 9 patients were alive and 10 had died (Table I). The patients who survived lived for 4 to 43 months (32.7 ± 6.0 S.D.) after the cessation of artificial pacing. The mean age of these patients at the time when they developed symptoms of myocardial infarction was 69.2 years (± 6.0 S.D.).

Of the 10 cases who were discharged from hospital but died later the interval between the cessation of pacing and their death averaged 14.8 months (± 12.4 S.D.). The mean age of these patients at the time when they developed myocardial infarction was 69 years. Table II shows the cumulative survival rate (8) in the patients who were not paced. In Fig. 1 the cumulative survival rate in the paced patients is compared with that in the patients who were not paced. It is seen that there was no statistically significant difference between the two groups in this respect during the first 3 months of the follow-up. Thereafter the difference became gradually more marked and after a follow-up time of 25-36 months it became statistically significant ($p < 0.01$). At that time 9 (29%) of the 31 paced patients were alive, the corresponding figure for the 373 patients who were not paced being 53%.

Causes of death

Out of the 22 patients who died, the mode of death could be determined in 21. Altogether 12 patients died suddenly. One patient died during the insertion of the pacemaker electrode although transient pacing was achieved. There was a drastic drop of blood pressure and he had five bouts of ventricular fibrillation. The post-mortem showed an infarct involving about 65% of the left ven-

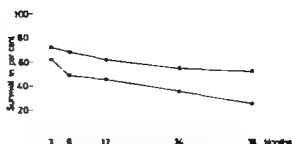


Fig. 1 Cumulative survival rate for the paced (○) and non-paced (□) patients with myocardial infarction treated during the same period.

tricle. Three other patients died suddenly during the treatment. During their stay in the CCU 4 of these 12 patients developed ventricular fibrillation which did not respond to DC shock. Another 4 patients died after a cardiac shock. One of the paced patients was readmitted to the Emergency Department about one month after her discharge from hospital. ECG showed recurrence of complete heart block. On admission blood pressure was unrecordable. She developed ventricular tachycardia and defibrillation resulted in asystole which did not respond to thumping of the chest. Two patients died of frank pulmonary oedema and another four of severe heart failure. One of the latter two patients developed bronchopneumonia shortly before his death.

Size of the infarct

In 26 cases GOT maximum could be assessed and was found to be on a range 277 (52-550) IU. Some of these patients had a past history of myocardial infarction. In the 13 cases in which a post-mortem was carried out and information on the area involved was available, the infarct involved 35-95% (mean 67%) of the left ventricle. In 11 of these 13 cases it was found also to involve a part of the posterior wall.

DISCUSSION

The incidence of complete heart block complicating myocardial infarction has been reported to range from 3 to 9% (3, 15, 16, 20, 23). Patients with this complication have a high mortality rate, 45-62% (3, 11, 16, 21). The majority of patients develop complete heart block shortly after the appearance of symptoms of

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THE EFFECT OF PHYSICAL EXERCISE ON THE WEDGED AND FREE HEPATIC VENOUS PRESSURE IN NORMAL MEN

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Abstract. Hepatic vein catheterization has been performed in 10 healthy male volunteers, 20-27 years old. Wedged and free hepatic venous pressures were repeatedly measured in supine position, at rest and up to 35 min after heavy bicycle exercise that reduced the hepatic blood flow to about one third. The mean pressure difference between the wedged and free catheter positions was in all subjects higher after exercise than before. The average value of 0.76 mmHg at rest increased to 1.92 mmHg after exercise. If the wedged hepatic venous pressure still measured the portal sinus pressure after exercise, the increased pressure gradient indicated a 2.5-3 times higher resistance to the portal blood flow over the hepatic sinusoids. This finding can be explained by increased water content and osmolality of the hepatocytes, causing mechanical obstruction of the sinusoids. The pathogenesis might be metabolic or hypoxic changes induced by the exercise.

The observation that the intravascular pressure on one side of a capillary bed can be measured from the other side by a catheter advanced into a wedged position was first reported with regard to the pulmonary capillaries (6, 10). This technique was soon applied also to the human hepatic circulation (9, 16). The similarity between wedged hepatic venous pressure and portal venous pressure has been established in man at various pressure levels both during operation (3, 13, 18) and in awake patients (17).

In the present report the free and wedged hepatic venous pressures were recorded both before and after heavy physical exercise in order to study the effect of exercise on the flow resistance of the sinusoidal capillaries.

MATERIAL AND METHODS

The material comprised 10 healthy male volunteers, some anthropometric data of whom are given in Table I. They

all passed clinical examination and had normal routine urine and blood tests, including liver function tests. None had history of previous liver, kidney, heart or lung disease. All subjects were studied in the morning 3 hours after very light breakfast. No premedication was given.

In the supine position right hepatic vein was percutaneously catheterized from an antecubital vein during TV fluoroscopy. By means of flexible stainless steel safety guide wire radio-opaque Wilson catheter (1.5, 2.3 mm) was placed as far peripherally as possible with the wedged position generally 4 cm from the lateral chest wall and the free position 3-5 cm more proximal. Intravascular pressures were measured by transducers (Bell & Howell, no. L 221 Working, Surrey, England) using an EMMA amplifier (SE lab, Fetham, Middlesex, England) and a UV writer (SE 3006, SE lab). Reference point for zero pressure was the midthoracic level at the insertion of the fourth rib at the sternum. Hepatic venous pressures were recorded during quiet breathing and amplified so that 1 mmHg of pressure corresponded to curve deflection of 1 cm. Whether or not properly wedged catheter position was obtained was judged by analysis of the undamped and damped curve as well as the anatomical position. For each measurement the pressure of the damped curve was integrated manually over 20-30 sec and stated to the nearest 1/10 of mmHg. It was observed that the easiest way to avoid pressure artefacts was to use straight catheters with fairly blunt ends.

Exercise was performed in the supine position on an electrodynamically braked bicycle ergometer (7). Two consecutive work loads of 20 min duration each were generally used, the second being twice as high as the first. They were chosen so as to correspond to the heaviest load the subject could be expected to complete. In four subjects (nos. 6, 7, 9 and 10) 15 min rest period was inserted between the two work loads.

The free and wedged hepatic venous pressures were repeatedly measured at rest before exercise as well as during the 30-35 min rest period following the heaviest load. They were also measured during the rest period after the first work load in the four subjects mentioned above.

Prior to the pressure recordings short teflon catheters

Table I Data obtained at rest and 3-35 min after supine bicycle exercise in 10 healthy male volunteers

I—first work load, II—second work load, WHV—wedged hepatic venous, FHV—free hepatic venous, number of individual observations, M_x —individual mean value of WHV-FHV at rest, M_{Ax} —individual mean value of WHV-FHV after exercise, \bar{x} —group mean value, S.D.—standard deviation, N—number of individuals

Subject no.	Age (y.)	Height (cm)	Weight (kg)	Work load (kpm/min)		Heart rate (beats/min)		Pressure at rest (mmHg)		Pressure after exercise (mmHg)						
				I	II	Rest	I	II	WHV FHV		WHV-FHV					
									WHV (n)	(M _R)	WHV (n)	(M _{AR})	M _{AR}	M _R	M _{AR} /M _R	
1	25	187	73	500	1 000	68	116	188 ^a	7.93	3	0.50	5.38	6	1.78	1.28	1.56
2	23	179	80	500	1 000	81	114	185 ^a	10.30	3	0.80	8.86	5	2.52	1.72	1.15
3	24	181	74	600	1 200	42	100	166	9.97	3	0.51	7.22	7	0.84	0.81	1.58
4	21	185	83	450	900	56	114	170	7.25	2	1.05	5.58	4	1.58	0.53	1.30
5	27	183	79	600	1 200	60	118	181	8.05	2	1.45	8.26	7	2.43	0.98	1.68
6	20	173	55	350	600	74	142	179 ^a	6.50	3	1.03	4.85	8	1.63	0.60	1.58
7	24	187	73	600	1 200	61	126	19 ^{ad}	9.9	1	0.5	8.60	2	2.95	2.65	9.81
8	26	193	100	—	1 000	63	—	138	9.43	3	0.73	9.25	7	2.04	1.31	2.79
9	23	185	73	550	1 000	56	118	193 ^a	11.75	2	0.65	9.95	8	2.63	1.98	4.05
10	27	175	72	500	1 000	61	104	161	9.03	4	0.55	5.73	3	0.80	0.25	1.45
\bar{x}	40	182.8	76.2	517	1 010	60	117	175	9.01	2.6	0.76	7.37	5.7	1.92	1.16	1.12
S.D.	4	6.0	11.2	81	179	8	12	17	1.58	0.8	0.34	1.85	2.1	0.73	0.78	2.35
N	10	10	10	9	10	10	9	10	10	10	10	10	10	10	10	10

Exercise terminated after 1. 19 min due to fatigue.

(11/1.4 mm) were introduced into an antecubital vein and a brachial artery for determination of estimated hepatic blood flow (EHBF) according to Bradley et al. (2) by constant infusion of Indocyanine Green dye (ICG). Repeated samplings from the artery and the hepatic vein were performed before, during and after exercise for determination of oxygen saturation and ICG concentration in plasma. These data will be reported separately and the methods are therefore only briefly mentioned here. Oxygen saturation as well as Hb concentration were analysed on CO oximeter (IL 182, Lexington, Mass., USA) and ICG concentration in plasma on a Hitachi 101 spectrophotometer with blank correction according to Nielsen (12). For the calculation of EHBF a correction for variations in plasma volume was made mainly as suggested by Hultman and Costenfor (8).



Fig. 1 Pressure differences between wedged hepatic venous (WHV) and free hepatic venous (FHV) catheter positions: rest (O) and after heavy supine bicycle exercise (Δ).

All blood drawn via the catheters prior to blood sampling was collected in syringes partly filled with heparinized saline and then immediately clamped into the hepatic vein. In no subject did the total blood loss exceed 250 ml.

Statistical calculations were performed according to Snedecor (13). A probability (*p*) level of <0.01 is called significant.

RESULTS

The individual and mean values for work loads, heart rates at rest and at the end of the work loads and hepatic venous pressures are given in Table I. In all but one subject the heart rate at the end of the heaviest load exceeded 160 beats/min.

The heaviest work load reduced the hepatic blood flow to about 35% of the value at rest, judged by the calculated values for hepatic blood flow and the changes in arterio-hepatic venous oxygen difference. The corresponding flow values 10, 20 and 30 min after exercise were about 80, 90 and 95% respectively.

After exercise the pressure fall from wedged to free hepatic vein catheter position did not change significantly during the period studied (Fig. 1). Therefore the individual mean values of all recorded pressures after exercise were used in further calculations. The average of the individual

mean values for wedged hepatic venous pressure decreased significantly from 9.01 mmHg before to 7.37 mmHg after exercise, but the free hepatic venous pressure decreased still more. Thus the difference between the wedged and free hepatic venous pressures increased in all subjects after exercise. The average of the individual means increased significantly from 0.76 mmHg at rest to 1.92 mmHg after exercise, or 2.5 times.

In the four subjects also studied during a rest period after the first work load the average pressure difference between wedged and free catheter position increased from 0.7 mmHg before to 1.1 mmHg after the first load and to 1.9 mmHg after the second.

The pressure difference between wedged and free hepatic vein at rest (M_1 Table I), after exercise (M_{12}), its change with exercise ($M_{12}-M_1$) and the quotient between the pressure difference after and before exercise (M_{12}/M_1) were studied as dependent variables in regression analyses. The independent variables used were absolute work load, heart rate as measure of relative work load, arterial blood pressure, arterio-hepatic venous oxygen difference and hepatic extraction of ICG. However with none of these variables recorded before, during or after exercise was a relationship of even probable significance observed.

DISCUSSION

Because of the dual blood supply of the liver and the complex vascular architecture of the sinusoids the question may be raised whether the hepatic venous pressure really equals the portal venous pressure during various conditions. The wedged hepatic venous pressure, however has been proved to correspond closely to portal venous pressure both in subjects with normal hepatic circulation (18) and in patients with cirrhosis (3, 13, 17). In the present study the wedged hepatic venous pressure was measured after exercise when possibly the hepatic artery blood flow formed a larger fraction of total hepatic blood flow than at rest. In the cirrhotics studied (3, 13, 17) intra-hepatic shunts and increased hepatic artery blood flow were certainly present to some extent, but still the wedged hepatic venous pressure equalled the portal venous. So far therefore, there are no indications that this is not also valid in normal subjects studied at rest and after exercise.

When the pressure gradients over the hepatic sinusoids, i.e. between wedged and non-wedged hepatic pressure were measured 10–30 min after exercise the hepatic blood flow was some 70 to 5% lower than at rest. When resistance is calculated as the fall in pressure over the vascular area divided by the blood flow the increase of the resistance to portal blood flow of the hepatic sinusoids after exercise should therefore be similar to the 2.5 times increase after exercise of the corresponding pressure gradient. An increase after exercise of the portal blood flow resistance of the hepatic sinusoids due to vasoconstriction is unlikely to occur so the observed increase would seem to be locally evoked. A swelling of the hepatocytes, hereby compressing the sinusoids, seems to be the most likely explanation. This would resemble the sinusoidal type of portal hypertension seen in the fatty liver and in toxic hepatitis (14). In working muscle cells water accumulates due to increased osmolarity especially after heavy exercise during anoxic conditions (11). A swelling of the hepatocytes after exercise due to increased water content should be a possible explanation of the observed increase in resistance.

In dogs with liver ischemia produced by clamping the hepatic artery and portal vein for 10–70 min (4) a subsequent increase of the ornithine carbamoyl transferase activity in serum (S-OCT) and a loss of OCT activity in the liver cells was observed. An increase of S-OCT has also been observed after surgery with general anesthesia in man and found to vary with the oxygen saturation in hepatic venous blood during operation (5), suggesting that the increase was caused by reduced hepatic blood flow and liver ischemia. After strenuous physical exercise in sitting position, when hepatic blood flow is more reduced than in supine position, an increase of S-OCT has also been observed in young healthy males (1), suggesting a release of OCT from the liver cells during exercise. Whether this is due to liver hypoxia during exercise or to a metabolic response to the work load is not possible to state. It seems possible, however that the same anoxic or normal metabolic changes induced by exercise that can evoke a release of OCT from the liver cells might also increase the water content of the hepatocytes and thereby increase the portal blood flow resistance of the hepatic sinusoids.

In this study of a small group of males we found that the increase of the resting to the post-exercise pressure gradient from wedged to free hepatic vein was not influenced by either the absolute (or relative) work load or by the degree of blood flow reduction during exercise.

ACKNOWLEDGEMENT

Supported by grant no. K.71-40X 3408-01 from the Swedish Medical Research Council.

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REGIONAL VARIATIONS IN HEPATIC BLOOD FLOW AND FUNCTION IN MAN

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Abstract. Four male patients have been studied with regard to arterio-hepatic venous extraction of oxygen and Indocyanine Green dye (ICG). Two patients with alcoholic cirrhosis of the liver had an atrophic right and hypertrophic left liver lobe. They also had higher extractions of oxygen and ICG over the right than over the left lobe, most pronounced for ICG. The two other patients, one with an extrahepatic biliary stasis and one with chronic pancreatitis, had livers of normal size and configuration. They displayed no regional differences in extraction of oxygen, but one of them had lower extraction of ICG over the right than over the left hepatic lobe. The findings in the patients with cirrhosis might be explained by regional variations in metabolic rate and hepatic cell function in relation to blood flow and possibly also by shunts from the portal vein to the hepatic veins of the left lobe. Total hepatic blood flow cannot be estimated with sufficient accuracy by the Bradley technique in patients in whom the hepatic extraction of the substance used is low and large regional variations in extraction may occur.

In patients with pronounced liver cirrhosis portal vascular changes have been observed intrahepatically at portography. Bergstrand (*) thus described decreased hepatographic density mottled appearance of the liver and narrow branches, ending abruptly or tapering irregularly. Aronson and Nylander (1) reported one patient having "extensive cirrhosis within the right liver lobe, with compensatory hypertrophy of the left one". Among patients with liver cirrhosis Wiechel et al. (2) observed similar vascular changes in all subjects, and in about half of them also atrophic right and hypertrophic left lobes with increased diameter of the left portal branch. It is, however

not yet known what functional impact these findings might have.

In the present study the hepatic extractions of oxygen and Indocyanine Green dye (ICG) were measured in blood samples drawn simultaneously both from a right and a left hepatic vein in two patients with alcoholic cirrhosis of the liver and in two others with extrahepatic biliary stasis and chronic pancreatitis, respectively.

MATERIAL AND METHODS

The material comprised four male patients, some data of whom are given in Table I. Patients 1 and 2 were male jaundiced alcoholics with pronounced liver cirrhosis and previous episodes of esophageal bleeding. They both had hypertrophic left liver lobe at scintigraphy. Direct selective portography via the reopened umbilical vein (3) proved patient 1 to have porto-caval shunting and typical findings of liver cirrhosis such as sparse ramifications of the right portal branch, which had smaller diameter than the left one. Patients 3 and 4 both had normal X-rays at portography with no porto-caval shunting. One of them was male abuser of alcohol, who was found to have an extrahepatic biliary stasis, the other had chronic pancreatitis.

The subjects were studied in the supine position after overnight fast without premedication. Both right and left hepatic veins were percutaneously catheterized in antecubital or femoral veins during TV fluoroscopy and the left or polyethylene catheters were placed as far peripherally as possible without being in a kinked position. During the quiet breathing no reflux of caval blood into the hepatic sinus (7) could have occurred in any subject.

After percutaneous catheterization of an antecubital vein and the left brachial artery the total hepatic blood flow was estimated according to the principle of Bradley

Table I Clinical and laboratory data, including values for hepatic arterio-venous (A-V) extraction of oxygen and ICG in four male patients (mean \pm S.D.)

WHV—wedged hepatic venous, FHV—free hepatic venous, Bra—brachial artery, RHV—right hepatic vein, LHV—left hepatic vein, EHBF—estimated hepatic blood flow. Figures within parentheses refer to number of observations.

Pat. no	Diagnosis	Age (y)	BSA (m ²)	BP (mmHg)		WHV—FHV	Oxygen saturation (%)			A-V oxygen extraction RHV/LHV
				WHV	FHV		Bra	RHV	LHV	
1	Alcoholic cirrhosis	39	1.83	24.8	11.8	13.0	93.2	57.6	63.1	1.19 ^{ns}
							0.3	1.2	2.1	0.08
							(5)	(5)	(5)	(5)
2	Alcoholic cirrhosis	67	1.88	30.0	6.3	23.7	98.2	73.8	78.2	1.21
							0.4	2.0	0.9	0.11
							(6)	(6)	(6)	(6)
3	Extrahepatic biliary stasis	62	1.87	13.9	8.5	5.4	90.2	52.0	51.7	1.81
							1.2	2.3	3.7	0.08
							(5)	(5)	(5)	(5)
4	Chronic pancreatitis	44	1.77	8.9	8.2	0.7	98.1	62.3	62.9	1.82
							0.2	2.0	1.0	0.05
							(4)	(4)	(4)	(4)

$p < 0.05$, $p < 0.01$ where p indicates the probability of the deviation of the quotient RHV/LHV from 1 being caused by random factors.

et al. (4). A priming dose of ICG was given into the antecubital vein, immediately followed by a constant infusion of ICG at a rate of 0.4–0.6 mg/min using a motor driven infusion pump and a calibrated syringe.

After an equilibration time of 30 min blood sampling started from the artery and the two hepatic veins. At intervals of 5–10 min five to six samples of 4 ml were drawn from each vessel, the arterial sample being collected 10–15 sec prior to the two venous ones. The ICG concentration in plasma was measured with individual calibration curves on a Hitachi 101 spectrophotometer, correction for blank density at 900 nm as performed to Nielsen (6) and total hepatic blood flow calculated from the average arterio-venous extraction and the average change in arterial concentration as suggested by Whicker et al. (9). The hematocrit was determined in all arterial samples.

From all three vessel blood samples of 3 ml for oxygen saturation and Hb concentration were collected after the ICG samples and analysed in CO oximeter (IL model 182, Lexington, Mass., USA). Intravascular pressures were measured by transducers (Bell & Howell L 221 Woking, Surrey England) using an EMMA amplifier (SE labo, Feltham, Middlesex, England) and UV writer (SE 3006, SE labo). Reference point for zero pressure was the mid-thoracic level at the insertion of the fourth rib at the sternum.

RESULTS

Both patients with alcoholic cirrhosis had significantly increased pressure gradients from wedged to free hepatic vein, indicating increased resist-

ance to portal blood flow through the liver. The patient with biliary stasis had a borderline increase while the fourth had a normal pressure gradient (Table I).

The oxygen saturation values of the two cirrhotics were consistently lower in all samples from the right than from the left hepatic vein. Thus the average quotient between the arterio-venous oxygen extractions over the right compared to the left hepatic vein were 1.19 and 1.21, respectively (Table I). In patients 3 and 4 no such differences were observed.

The hepatic extractions of ICG in the two cirrhotics were also consistently higher in the right than in the left hepatic lobe, as measured in their efferent vessels. The average quotient between the extractions observed in the right compared to the left hepatic vein blood was 1.51 and 1.44 respectively. In patient 4 no right-to-left difference was observed, but in patient 3 a significant but reversed difference was noted, with an average quotient between right and left hepatic vein extraction of 0.80.

DISCUSSION

Although only four patients have been examined so far the results seem to justify a presentation in order to initiate further studies.

A-V ICG extraction		EHBF (l/min) calculated from ICG concentration in		
RHV (%)	LHV (%)	RHV/LHV	RHV	LHV
121	8.2	1.51	1.64	2.30
111	1.0	0.26		
09	(6)	(6)		
127	13.2	1.44	0.77	1.11
118	1.4	0.35		
06	(6)	(6)		
162	20.3	0.80*	2.47	1.97
110	1.7	0.07		
05	(5)	(5)		
132	10.5	1.04	1.24	1.27
12	7.1	0.09		
04	(4)	(4)		

In the two cirrhotics studied the hepatic extractions of oxygen as well as ICG were significantly higher over the right than over the left lobe. The correlation between these findings and the differences between the corresponding parts of the liver as seen at portography have to be more thoroughly investigated, as well as possible variations within each lobe.

Many factors may contribute to the heterogeneity, such as intrahepatic shunts between portal branches and hepatic veins and/or between hepatic arteries and hepatic veins, regional differences in portal ein and hepatic artery blood flow differences in oxygen saturation of the two portal branches, differences in metabolic rate in relation to blood flow and differences in hepatic cell function with regard to ICG extraction. The present study does not give conclusive evidence as to which of these possibilities is most likely but some of the factors will be discussed. Intrahepatic shunts from the hepatic artery to hepatic eins in the left liver lobe cannot be the only cause of the present findings, as in such case similarly reduced extractions of ICG and oxygen would be expected over the left lobe. If on the contrary the shunts were mainly from portal eins to hepatic eins, the present data could be explained in this way. However the amount of the intrahepatically shunted blood has been found

to correlate well with the degree of cirrhotic changes (5), and in the present study the atrophic cirrhotic changes were most pronounced in the right lobe of the first patient according to the portography. This was also observed by Waechel et al. (8) in patients with liver cirrhosis. Although oxygen saturation in blood from right and left portal branches was not measured in these subjects, no differences should be expected according to our previous observations in a few patients with these portographic findings.

Bradley (3) has described the variability in ICG extraction that can exist when using different catheter positions in right hepatic eins. In the study by Winkler et al. (9) in one patient with a mild liver cirrhosis and seven subjects without liver disease, two usually right-sided hepatic eins were catheterized and the liver extraction of five different substances was simultaneously studied. Significant differences in extraction between the two hepatic catheters were seen in some subjects, but generally only for one or two of the substances tested. These differences were not simultaneous with variations in oxygen saturation of the hepatic venous blood, and thus they were regarded as signs of variations in metabolic function rather than in blood flow. The data for patient 3 in the present study with a higher extraction of ICG in the left than in the right lobe but the same oxygen saturation values, are in good agreement with their findings. Our observations in the two patients with alcoholic cirrhosis, however seem to be quite different.

Finally the present study illustrates the hazard of estimating the total hepatic blood flow by the Bradley technique in patients in whom the hepatic extraction of the substance used is low and large variations in extraction may occur.

ACKNOWLEDGEMENTS

Supported by grant no. K71-40X-3408-01 from the Swedish Medical Research Council and grants nos. 179-872-02P, 354-870-02P, 377-872-02P and 633-872-01P from the Swedish Cancer Research Foundation.

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Table 1 Clinical and laboratory data, including values for hepatic arterio-venous (A/V) extraction of oxygen and ICG in four male patients (mean \pm S.D.)

WHV = wedged hepatic venous, FHV = free hepatic venous, BrA = brachial artery, RHV = right hepatic vein, LHV = left hepatic vein, EHBF = estimated hepatic blood flow. Figures within parentheses refer to number of observations.

Pat. no.	Diagnosis	Age (y)	BSA (m ²)	BP (mmHg)		WHV-FHV	Oxygen saturation (%)			A/V oxygen extraction RHV/LHV
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1	Alcoholic cirrhosis	39	1.83	24.8	11.8	13.0	93.2 0.3 (5)	57.6 1.2 (5)	63.1 2.1 (5)	1.19 ^{ns} 0.08 (5)
2	Alcoholic cirrhosis	67	1.88	30.0	6.3	23.7	98.2 0.4 (6)	73.8 3.0 (6)	78.2 0.9 (6)	1.21 ^{ns} 0.11 (6)
3	Extrahepatic biliary stasis	62	1.87	13.9	8.5	5.4	90.2 1.2 (5)	52.0 2.3 (5)	51.7 3.7 (5)	1.01 0.08 (5)
4	Chronic pancreatitis	44	1.77	8.9	8.2	0.7	98.1 0.2 (4)	62.3 2.0 (4)	82.9 1.8 (4)	1.02 0.05 (4)

$p < 0.05$ $p < 0.01$ where p indicates the probability of the deviation of the quotient RHV/LHV from 1 being caused by random factors.

et al. (4). A priming dose of ICG was given into the antecubital vein, immediately followed by a constant infusion of ICG 1 at a rate of 0.4–0.6 mg/min using a motor driven infusion pump and a calibrated syringe.

After an equilibration time of 30 min blood sampling started from the artery and the two hepatic veins. At intervals of 5–10 min five to six samples of 4 ml were drawn from each vessel, the arterial sample being collected 10–15 sec prior to the two venous ones. The ICG concentration in plasma was measured with individual calibration curves on Hitachi 101 spectrophotometer. Correction for blank density at 900 nm was performed according to Nielsen (6) and total hepatic blood flow as calculated from the average arterio-venous extraction and the average change in arterial concentration as suggested by Winkler et al. (9). The haematocrit was determined in all arterial samples.

From all three vessels blood samples of 5 ml for oxygen saturation and Hb concentration were collected after the ICG samples and analysed in CO counter (IL model 182, Lexington, Mass. USA). Intravascular pressures were measured by transducers (Bell & How II L 221, Woking, Surrey England) using an EMMa amplifier (SE lab, Feltham, Middlesex, England) and UV writer (SE 3006, SE lab). Reference point for zero pressure was the mid-thoracic level at the insertion of the fourth rib at the sternum.

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The hepatic extractions of ICG in the two cirrhotics were also consistently higher in the right than in the left hepatic lobe as measured in their efferent vessels. The average quotient between the extractions observed in the right compared to the left hepatic vein blood was 1.51 and 1.44 respectively. In patient 4 no right-to-left difference was observed, but in patient 3 a significant but reversed difference was noted, with an average quotient between right and left hepatic vein extraction of 0.80.

DISCUSSION

Although only four patients have been examined so far the results seem to justify a presentation in order to initiate further studies.

ESTIMATIONS OF LOBAR HEPATIC BLOOD FLOWS AND EXTRACTIONS IN SEVERE LIVER CIRRHOSIS

Preliminary Report

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Previously suggested methods for determination of portal vein and hepatic artery blood flow (1-3) cannot be used in patients with advanced liver cirrhosis. This is due both to the marked regional variation in hepatic extraction of oxygen and indocyanine Green dye (ICG) that can be observed in these patients (4) which renders the Bradley technique inadequate for measuring total hepatic blood flow and to possible prehepatic portal-caval shunting of blood and indicator. The present study describes a technique for determination of the various hepatic blood flows in these patients.

MATERIAL AND METHOD

The material comprised one female alcoholic with severe liver cirrhosis, ascites and moderate jaundice (Table 1). At selective portography the left portal branch was larger than the right, and the left hepatic lobe was larger than the right. There was also marked shunting of blood from the splenic to the inferior caval vein. The portal venous pressure was increased (33 mmHg), the free hepatic venous pressure being normal (10 mmHg). After their informed consent to males with extrahepatic biliary stases were also studied after diagnostic portography had been performed. Their portal venous pressures were 13 and 15 mmHg, respectively. Neither of them had liver cirrhosis or shunt.

Catheters. As previously described (7) a large outer catheter as in local anaesthesia inserted in the umbilical vein to the distal end of the left portal branch. Within this outer catheter a 2.0/2.5 mm wide teflon one was located into the splenic vein. Then guided, 1.05/1.40 mm wide fluor-ethylene-propylene spray catheter with occluded end and four side holes was also positioned there. The 2.0/2.5 teflon catheter was removed and beside the infusion catheter a 1.0/1.3 mm wide teflon one was introduced through the outer catheter into the right portal branch using suitable adapters. Additional catheters were percutaneously inserted into peripheral artery, peripheral vein and right hepatic vein. In the patient with cirrhosis one additional right and one left hepatic vein were also catheterized.

Infusion. Through the spray catheter 0.5-0.5 mCi ^{125}Xe (Atomasegyl, Sönderby, Sweden) in 100 ml physiological saline was repeatedly infused, first into the splenic vein at rate of 13 ml/min, later at rate of 27 ml/min into the middle of the main portal trunk and finally into the first part of the left portal branch. To obtain equilibrium between indicator activity in blood and hepatic tissue in 1-2 min both injection corresponding to about 1 min of infusion preceded the constant infusion.

Sampling and measurement. During the infusions air free blood samples were drawn in duplicate in 2 ml heparinized plastic syringes from the two portal branches, the hepatic veins and the peripheral artery. The ^{125}Xe activity in the blood samples was measured within the syringes in a well scintillation detector coupled to Picker Digital Rate Computer (5). The blood flow in the main portal trunk was calculated from infusions into the splenic or portal veins as

$$F_{\text{portal vein}} = F_{\text{infusion}} \frac{(C_{\text{infusion}} - C_{\text{portal vein}})}{(C_{\text{portal vein}} - C_{\text{arterial}})}$$

where F = flow in ml/min and C = activity of ^{125}Xe . Blood flow in the left portal branch was similarly calculated from infusion into this vessel. Hepatic artery blood flows in the right and left hepatic lobes were calculated from lobar portal vein flows and the dilution of indicator from portal to hepatic vein blood.

ICG was constantly infused into the peripheral vein and determined spectrophotometrically by standard procedures in arterial and hepatic vein samples. Oxygen saturation was measured in an IL 182 CO analyzer.

RESULTS

Determined and derived flow values are given in Table 1. The left portal branch contributed 47% of total portal venous flow in the cirrhotic patient compared to 4 and 22% respectively in the two others without signs of liver cirrhosis. In the latter two patients the right hepatic vein blood as regarded as representative of the whole liver. The right portal branch flow values were about equal in all three patients.

Table 1 Clinical data and splanchnic flow values of one female alcoholic (no. 1) with severe cirrhosis of the liver and two males with extrahepatic biliary stasis (nos. 2 and 3)

Pat. no.	Age (y)	BSA (m ²)	Portal vein flow (l/min)			Hepatic artery flow (l/min)			Hepatic vein flow (l/min)		
			Right branch	Left branch	Total	Right lobe	Left lobe	Total	Right lobe	Left lobe	Total
1	49	1.66	0.63	0.56	1.19	0.35	1.19	1.54	0.98	1.75	2.73
2	66	1.80	0.35	0.17	0.72			0.50			1.22
3	61	1.88	0.80	0.23	1.03			0.25			1.28

In the cirrhotic patient the hepatic artery contribution was 36%, in the right lobe, 37 and 34% respectively in the two veins, but 68% in the left lobe. In patients 2 and 3 the hepatic artery flows were 41 and 20% respectively of total hepatic flow.

Values for oxygen and ICG content in blood are only given to patient 1 to illustrate the complex function of the cirrhotic liver (Fig. 1). The hepatic extraction of both oxygen and ICG were lowest in the right superior and highest in the right inferior hepatic vein.

DISCUSSION

With infusions into the splenic vein and the main portal trunk adequate mixing of indicator and blood was obtained, as indicated by xenon activities varying less than 10% in the two portal branches (5). During infusions into the left portal branch no indicator activity above arterial background was recorded from right hepatic vein blood. Mixing within the left portal branch was controlled in patient 3 by repeated flow determination with a slightly changed catheter position, giving a value within 10% of the first one. Thus the present variation of segmental venous flow determination should give satisfactory results, although lower infusion rates were used, than when caval

and iliac vein flows were measured with specially designed spray catheter (2).

The failure of the Bradley technique to measure total hepatic blood flow in cirrhotics is illustrated by the marked variations in extraction between the hepatic veins in the patient studied. Corresponding calculated total hepatic flows varied between 1 and 5 l/min. With the present method detailed analysis of the complex hepatic circulation in this cirrhotic patient was possible. The large left hepatic lobe had not only doubled its portal venous blood supply but also increased its hepatic artery blood supply 3-4 times above predicted normal values. This increase in hepatic artery contribution in the left lobe, however, cannot be the cause of the observed variations in hepatic extraction, as great differences occurred between the two right hepatic veins, in which the arterial contribution was equal. Neither can shunting of blood from the hepatic artery or the portal vein to the hepatic vein explain these differences, as the variations in arteriovenous extraction of ICG were more marked than those of oxygen and also than the variations in portal-hepatic venous extractions of oxygen. Thus great regional differences in metabolic activity in relation to blood flow seemed to be present, perhaps related to regional variations in cell regeneration.

ACKNOWLEDGEMENT

Supported by grant no. K.71-40X 3408-01 from the Swedish Medical Research Council and grants nos. 179-K70-40X, 179-B72-02P, 377-B72-02P from the Swedish Cancer Research Foundation.

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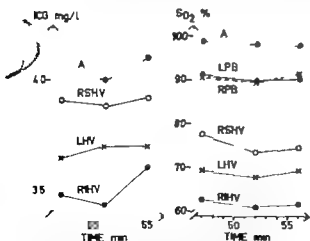


Fig. 1 Oxygen saturations (SO_2) and blood concentrations of ICG during constant infusion of ICG in patient 1. A=brachial artery, RHV=LHV and RSHV=right inferior left and right superior hepatic vein, LPB=left, RPB=right portal branch.

CONFIRMATION OF POSTCHOLECYSTECTOMY BILIARY DYSKINESIA BY ELEVATION OF SERUM TRANSAMINASES (GOT AND GPT) AFTER INJECTION OF MORPHINE?

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Abstract. In 39 cholecystectomized patients and 62 patients without previous cholecystectomy the effect of 1 m. injection of morphine on the serum concentrations of bilirubin, alkaline phosphatase, GOT, GPT, LDH and amylase has been studied. Most of the cholecystectomized patients in whom the injection of morphine provoked reaction of abdominal pain showed increased serum activity of both GOT and GPT 8 hours after the injection of morphine. The results suggest that this "morphine-enzyme-pain" provocation may be helpful in differentiating dyskinesia of the sphincter Oddi from other post-cholecystectomy abdominal complaints.

Recurring or persisting complaints after cholecystectomy due to a motoric or functional disorder of the sphincter Oddi, can be mimicked by the administration of morphine (3, 5, 22). Since 1957 several authors have suggested that this so-called dyskinesia of the sphincter Oddi can be objectivated by studying changes in the serum levels of standard liver function tests (serum glutamic oxalacetic transaminase (GOT), alkaline phosphatase, lactic acid dehydrogenase (LDH), serum glutamic pyruvic transaminase (GPT)), after the administration of morphine. These reports, however deal with a small number of patients (9, 15, 17).

The purpose of the present investigation was to study in a larger group of patients changes in standard liver function tests after administration of morphine in order to see whether these changes might yield an objective criterion for ascribing the morphine-provoked pain to the biliary tract.

MATERIAL AND METHODS

Patients

Of total of 101 patients studied 38 had undergone cholecystectomy of whom 25 had persisting or recurrent complaints after operation.

Of the 38 non-cholecystectomized patients 42 had abdominal complaints, which were of unknown origin in 11 and were diagnosed in the other 31 as cholelithiasis (8 pts), peptic ulcer (9 pts), hiatal hernia (4 pts), achalasia (1 pt), postgastroctomy complaints (6 pts), liver cirrhosis (2 pts) and diverticular coli (1 pt).

A painful sensation after administration of morphine occurred in patients both with and without previous cholecystectomy. For the purpose of this study the patients were classified in four groups: 1. 18 of the 39 cholecystectomized patients injection of morphine provoked painful sensation (group 1), while in the remaining 21 it did not (group 2). From the 38 non-cholecystectomized patients 12 reacted with morphine-provoked pain (group 3) while the remaining 26 did not (group 4).

Morphine-enzyme-pain provocation test (VEP test)

Patients were informed neither about the drug administered nor about the possibly occurring reactions. A fasting venous blood sample was drawn at 8 a.m. for determination of the serum levels of bilirubin, alkaline phosphatase, amylase, GOT, GPT and LDH. Morphine was then administered in dose of 10 mg i.m. and further blood samples were collected after 2, 4, 8 and 4 hours for determination of the same variables. The patients remained fasting until 0.30 p.m. and during the test period they were asked to report any effect they thought to be due to the injection. If discomfort, pain or colic occurred, the nature of these complaints was evaluated and the patient was asked whether he "recognized" the pain. The changes in the serum levels of bilirubin and enzymes studied are expressed in percentage of the "zero value" obtained before morphine administration.

RESULTS

Relation between provoked pain and biochemical changes

Fig. 1 shows that the average increase of both bilirubin and of alkaline phosphatase, GOT, GPT and LDH—especially GOT and GPT—is higher in postcholecystectomy patients in whom injection of morphine provoked a pain reaction (group 1) than in all other groups of patients.

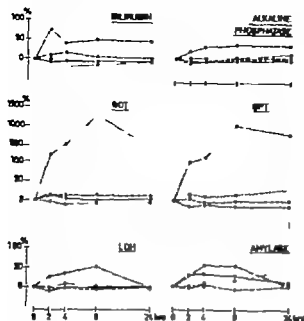


Fig. 1 Mean changes of the serum levels of bilirubin and enzymes (expressed as percentage of the "zero values" before injection of morphine) 2, 4, 8, and 24 hours after the injection of morphine in the four patient groups. — group 1, --- group 2, - - - group 3, ——— group 4.

As the maximal increase occurred mostly 8 hours after the injection of morphine, only 8-hour values are further analysed. The differences regarding these average increases of bilirubin, alkaline phosphatase, GOT, GPT and LDH between group 1 and the other groups are significant, although at different levels (Table I), with the exception of the average increase of bilirubin in groups 1 and 2.

There was no significant difference in the average change of the values of amylase between the four groups studied. GOT and GPT showed the

Table I *P*-values (Student's *t*-test) of the differences of average increase of bilirubin and enzymes studied (8 h after injection of morphine) between group 1 and groups 2, 3 and 4

	Group 1					
	Bil- rubin	Alka- line phos- phatase	Amy- lase	GOT	GPT	LDH
Group 2	<.10	<.001	<.25	<.01	<.02	<.005
Group 3	<.05	<.01	<.25	<.05	<.05	<.05
Group 4	<.005	<.0005	>.25	<.005	<.005	<.0005

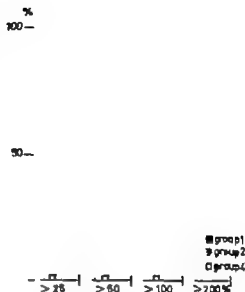


Fig. 2 Combined elevation of GOT and GPT. Percentage of patients in each group in which 8 h after morphine both enzymes showed an increase of >25%, >50%, >100% or >200%.

greatest average increase. Although there is a significant average increase in alkaline phosphatase and LDH, the absolute change in enzyme levels is rather small and therefore clinically not well applicable. Therefore it was investigated whether the combination of the changes of the serum levels of both GOT and GPT might differentiate between the individual patients of group 1 and those of the other three groups. The frequency in the four groups of an increase of both GOT and GPT by more than 25, 50, 100 and 200% is shown in Fig. 2. It will be seen that an increase of both GOT and GPT by more than 100% was very often found in the patients of group 1 but never in group 2, and rarely (only in one patient of group 4) in non-cholecystectomized patients with or without pain after injection of morphine.

Relation between anamnestic pain, morphine-provoked pain and changes in the serum levels of GOT and GPT

To evaluate the possibility of using the above mentioned findings as a diagnostic aid in dyskinesia of the sphincter Oddi, the relation between spontaneous abdominal pain, morphine-provoked pain and increase of serum transaminases of more than 100% was studied (Table II).

Table II. Relation between anamnestic pain, morphine-provoked pain and elevation of both GOT and GPT

Character of spontaneous upper abdominal pain complaints (no. of pts.)	Character of pain reaction provoked by morphine (no. of pts.)	Elevation of both SGOT and SGPT with more than 100% (no. of pts.)
After cholecystectomy		
Colic pain 8	Colic pain 5	5
	No pain 3	
Pain 17	Colic pain 2	2
	Pain 6	4
	Dubious pain 1	
	No pain 8	
No pain 14	Colic pain 2	2
	Pain 1	
	Dubious pain 2	
	No pain 9	
No cholecystectomy		
Colic pain 5	Colic pain 5	
Pain 32	Pain 9	
	Dubious pain 1	
	No pain 26	1
No pain 25	Pain 1	
	No pain 24	

After cholecystectomy an increase of both GOT and GPT was very often (but not always) found in patients with spontaneous upper abdominal pain if similar pain could be provoked by the administration of morphine. An increase of both GOT and GPT was also found in cholecystectomized patients without spontaneous complaints if morphine induced a colic. An increase of both GOT and GPT of more than 100% was not observed either in cholecystectomized patients if no pain was provoked by morphine or in patients with a gall bladder even if the anamnestic pain could be provoked by the administration of morphine.

DISCUSSION

The present results indicate that pain provoked by morphine is generally accompanied by an increase of both GOT and GPT in cholecystectomized patients, but not in patients with a gall bladder. These findings corroborate previous reports (9, 11, 17) but are also new as the combined behaviour of both GOT and GPT has never before been examined in such a large series of cholecystectomized and non-cholecystectomized patients.

Morphine is known to cause spasm of the

sphincter Oddi (14) and a concomitant rise of intrabiliary pressure in postcholecystectomized patients (7, 8, 12). Our findings are explicable by the fact that this increased biliary pressure, which lasts approximately 30 min (12, 19), causes some damage to the liver parenchyma, enhancing (6) the leakage of intracellular enzymes into the plasma. This produces a considerable percental increase in the serum level of GOT and GPT (situated mainly in the cytoplasm) and possibly of the liver fraction of LDH, but this was not determined separately (20). As GOT and GPT elevations are mainly caused by cell damage (20) and bilirubin and alkaline phosphatase (11) are indicators of disturbance of the secretory and synthetic liver cell function, one might assume that the short-lasting rise in intrabiliary pressure results in only transient cell damage.

Further one might conceive that the presence of a normally distensible gall bladder may prevent an increase of the intrabiliary pressure after administration of morphine, and thus cell damage. However this explanation must be incomplete as it does not fit very well with the strange behaviour also known in the literature (4, 10, 13, 18, 19, 21), of serum amylase. After injection of morphine, amylase increases or decreases without correlation with either presence of the gall bladder or provoked pain.

Pain provocation by morphine is often used as a clinical test to ascertain that postcholecystectomy complaints are due to biliary dyskinesia (3). However morphine may provoke upper abdominal pain both in patients with and without postcholecystectomy complaints (Table II) abdominal pain which is provoked by morphine may be due not only to spasm of the sphincter Oddi but also to spasm of the bowel (1, 2), e.g. in diverticulosis. In this respect the addition of serum enzyme determination to the morphine pain provocation test is helpful as it will differentiate between morphine-provoked pain originating from spasm of the sphincter Oddi and morphine-provoked pain from the bowel (16).

Thus a positive MEP test is compatible with dyskinesia of the sphincter Oddi when the postcholecystectomy abdominal pain is brought about by the injection of morphine and accompanied by an increase of more than 100% of both GOT and GPT 2 hours after administration of the drug.

Two patients are reported (15) with a post cholecystectomy syndrome in whom morphine provoked a pain reaction and increase of the serum level of GPT and LDH, and in whom at operation a hypertrophic sphincter Oddi was found. Of our patients with complaints after cholecystectomy 6 were submitted to surgery of the biliary tract, 2 of them had a positive MEP test and at operation a thickened papilla Vateri that was hard to probe, the other 4 patients had a negative test and at operation a non-hypertrophic papilla Vateri that was easy to probe. In another patient with recurrent colic after cholecystectomy on whom no MEP test was performed, but who had an increase of both GOT and GPT after a spontaneous colic during hospitalization, a thickened papilla Vateri was also found.

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THE UPTAKE OF GALLIUM-67 IN EUTHYROID PATIENTS WITH MULTINODULAR GOITRE

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Abstract. The presence of a carcinoma in multinodular goitre of an euthyroid patient cannot be ruled out by means of radioactive iodine and technetium scanning. Gallium-67 (Ga-67) citrate, radiopharmaceutical with high tumour affinity has been given intravenously to 13 patients. Five patients showed positive gallium scan of the thyroid gland, 3 of them had thyroid carcinoma, 1 leucosarcoma and 1 thyroiditis. Two other patients showed uptake of radioactive gallium in metastasis of thyroid carcinoma in the skull. The remaining 6 patients showed negative gallium scan of the thyroid gland, 2 of them had small thyroid carcinoma. Scanning with Ga-67 seems to be useful in the preoperative diagnosis of thyroid carcinoma.

A multinodular goitre in euthyroidism is usually of a benign nature. However the possibility of the existence of a thyroid carcinoma must never be excluded. Sometimes the history of the patient and the physical examination reveal malignancy but none of the usual criteria is wholly satisfactory. The problem cannot be solved by thyroid scanning following the administration of iodine 131 (I-131) or technetium 99 m (Tc-99 m) because the result is a cold nodule in both benignancy and malignancy. Edwards and Hayes (1) demonstrated that, after i.v. injection of gallium-67 (Ga-67) as carrier-free gallium citrate, the Ga-67 is localized in a variety of non-osteous and skeletal malignant tumours allowing detection by scanning. It is also concentrated in normal tissue, in the liver the spleen, the axial skeleton and the parotid gland. However gallium concentrates preferably in viable tumour cells, where it has been demonstrated in the cytoplasm of the living malignant cells. The concentration in tumour tissue is maximal 24 hours after the administration of Ga-67 and remains constant for a period of 6

days (2, 3). Gallium does not concentrate in fibrotic and necrotic tissue. Gallium is excreted in urine and faeces.

We decided to investigate the usefulness of Ga-67 in detection of carcinoma of the thyroid gland.

PATIENTS AND METHODS

Thirteen patients were assigned, 11 with an euthyroid multinodular goitre and 2 with hypothyroid goitre. Thyroid function was defined according to the clinical features, the serum protein-bound iodine, T₄ resin uptake test, T₄ competitive protein binding and I-131 tracer study. In all cases surgical treatment was decided because of obstruction symptoms and suspicion of malignancy.

These patients were scanned after administration of the following three radiopharmaceuticals.

T-99 m. Scanning was performed 1 hour after i.v. administration of 1 mCi as Tc-99 m pertechnetate.

I-131. Scanning was performed 6 hours to 3 days after oral administration of 40-100 mCi as NaI 131.

Ga-67. Scanning was performed 2-3 days after i.v. administration of 1.5-2 mCi as Ga-67 citrate (Philips-Duphar, the Netherlands). Scintigrams were made with a rectilinear scanner (Magne Scanner V. Picker) and a γ -camera (Nuclear Chicago). The activity found in the Ga-67 channel was not due to residual activity from the earlier I-131 study. The results of the histological examination of the resected thyroid tissue are summarized in Table I.

CASE REPORTS

Patient 1

Since 1968 this 46-year-old man had noticed progressive enlargement of the thyroid gland. In the year 1971 a tumour of about 10 cm with hard areas was palpable. This tumour did not show any change after administration of Tc-99 m. The Ga-67 scan was

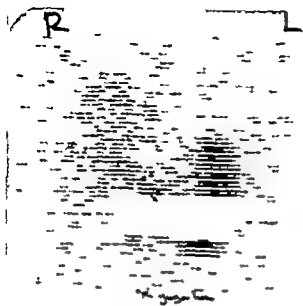


Fig. 1 Patient 3. Thyroid scan showing I-131 uptake in the left lobe and the isthmus.

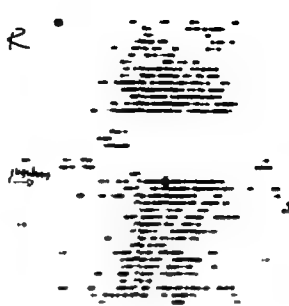


Fig. 2 Patient 3. Thyroid scan showing Ga-67 uptake in the right lobe with concentration behind the sternum.

Patient 2

Since 1969 this 66-year-old woman had noticed a progressive enlargement of the thyroid gland. Two years later she developed complaints of hoarseness and respiratory discomfort. Besides enlargement of the left lobe of the thyroid gland several metastases were found. No pathological lymph glands could be detected, but the left recurrent nerve was paralysed. X-ray examination showed a broadening of the upper mediastinum and a number of circular shadows in both lungs. Tc-99 m thyroid scanning showed no uptake in the left lobe. A Ga-67 uptake could

not be detected in this lobe, but could be demonstrated in the right lobe and in the circular shadows in the lungs. Thyroidectomy was impossible because of extensive infiltration. Tissue obtained from biopsy of the left lobe of the thyroid gland from a region of non-radioactivity following the administration of Ga-67 consisted of sclerotic necrosis and a few cells of thyroid carcinoma.

Patient 3

In 1968 this 61-year-old man had noticed progressive enlargement of the thyroid gland. In 1970 the patient suffered respiratory discomfort. Part of the greatly enlarged multinodular thyroid gland was projecting through the upper aperture of the thorax. No pathological lymph glands were found. X-ray studies revealed polycyclic broadening of the right upper mediastinum. The I-131 thyroid scanning showed a peak located in the left lobe and in the isthmus (Fig. 1). A uptake of I-131 was not seen in the right lobe of the thyroid gland or in the retrosternal area, but Ga-67 showed distinct concentration in this right lobe with concentration behind the sternum (Fig. 2).

Patient 4

In 1953 a destruction of the sacral bone of this 64-year-old woman was found, which appeared to be due to metastasis from thyroid carcinoma. However, no palpable abnormalities of the neck could be detected. After thyroidectomy at this time there appeared to be an adenocarcinoma of the thyroid gland. No thyroid suppression therapy was prescribed. Twenty years later the patient had an osteolytic metastasis on the left side of the skull which showed uptake of I-131 Tc-99 m and Ga-67. No uptake of these nuclides was seen in the neck region.

Table I Comparison of the results of the gallium scanning and the histological appearance of the thyroid tissue

Pat. no.	Sex	Age (y)	Pathological diagnosis	Ga uptake in thyroid tissue
1	♂	46	Follicular adenocarcinoma	+
2	♀	66	Thyroid carcinoma	+
3	♂	61	Follicular adenocarcinoma	+
4	♀	64	Adenocarcinoma	+
5	♀	42	Follicular adenocarcinoma	+
6	♀	63	Follicular adenocarcinoma	—
7	♂	18	Papillary adenocarcinoma	—
8	♀	54	Lemosarcoma	+
9	♂	32	Lymphocytic thyroiditis	+
10	♀	55	Colloid adenomatous goitre	—
11	♂	58	Colloid adenomatous goitre	—
12	♀	55	Colloid adenomatous goitre	—
13	♀	64	Colloid adenomatous goitre	—

In skull metastasis.

Patient 5

In 1952 this 42-year-old woman was radiated on the neck area because of a goitre. In 1964 she had noticed progressive enlargement of a multinodular goitre. After an ectomy in 1971 the thyroid gland appeared to contain follicular adenocarcinoma. A metastasis in the skull showed uptake of I-131 and Ga-67. A metastasis in the liver showed distinct uptake of I-131, but decreased uptake of Ga-67.

Patient 6

Since early youth this 63-year-old woman had goitre without complaints. In 1971 she revealed progressive stridor. The multinodular goitre was greatly enlarged with protrusion through the upper aperture of the thorax. X-ray studies revealed broadening of the upper mediastinum and considerable narrowing of the tracheal tube. I-131 scanning of the neck region showed an irregular uptake in the thyroid gland and no uptake in the thoracic enlargement of the thyroid gland. Gallium uptake was not detectable.

Patient 7

In 1970 pathological lymph gland was observed on the right side of the neck of this 18-year-old man. It appeared to be metastases of papillary adenocarcinoma of the thyroid gland. Scanning of the neck region showed thyroid gland with normal uptake of T-99 m and no uptake of Ga-67. After thyroidectomy the thyroid gland appeared to contain papillary carcinoma.

Patient 8

In 1971 this 54-year-old woman observed progressive enlargement of a nodule in the left lobe of the thyroid gland. No pathological lymph glands could be detected. The left recurrent nerve was found to be paralyzed. The palpable nodule in the thyroid gland did not show I-131 uptake, but it did show Ga-67 uptake.

Patient 9

In 1971 this 32-year-old man developed nodular enlarged thyroid gland and tender swollen right parotid gland. The patient gained weight and was inert and sleepy. The thyroid gland showed decreased I-131 uptake. However, Ga-67 concentrated in the thyroid gland and in the right parotid gland. The histological appearance of three specimens obtained by biopsy from different parts of the thyroid gland was the same: extensive infiltration with plasma cells and reactive areas of lymphocytes without any signs of malignancy. Biopsy from the right parotid gland showed parotid tissue with lymphoplasmocellular infiltration.

Patient 10

In 1945 this 35-year-old woman underwent subtotal thyroidectomy. In 1970 the thyroid gland was again found to be enlarged. A palpable nodule in the thyroid gland did not show uptake of T-99 m, I-131 or Ga-67.

Patient 11

In 1969 this 58-year-old man noticed progressive enlargement of the thyroid gland. A palpable nodule in the right lobe did not show uptake of Tc-99 m or Ga-67.

Patient 12

In 1971 this 55-year-old woman noticed an enlargement of the thyroid gland. No pathological lymph glands were found. The right recurrent nerve was found to be paralyzed. A palpable nodule in the right lobe of the thyroid gland did not show uptake of T-99 m, I-131 or Ga-67. After total hemithyroidectomy the right lobe appeared to be colloid adenomatous tissue without signs of malignancy. The right recurrent nerve is not infiltrated, so the origin of the paralysis remained uncertain.

Patient 13

In 1935 this 64-year-old woman underwent subtotal thyroidectomy. In the following years she received no thyroid substitution therapy. Meanwhile large multinodular goitre was developing. In 1970 the patient revealed an inspiratory stridor. Pathological lymph glands could not be found. X-ray examination showed broadened upper mediastinum and considerably narrowed tracheal tube. Irregular I-131 uptake was seen in the thyroid gland and in the upper mediastinum. The Ga-67 scan was negative.

DISCUSSION

Preoperatively 13 patients with an euthyroid multinodular goitre were investigated by gallium scanning. The interpretation of the gallium uptake in the neck region was disturbed by the concentration of Ga-67 in the cervical spine. It was obvious that the age of the patient is also a factor of importance. The gallium uptake appeared to occur much more easily in a young skeleton than in an older one.

Out of 5 patients with a positive gallium scan of the thyroid gland 3 had a thyroid carcinoma, 1 a leiomyosarcoma and 1 a lymphocytic thyroiditis. Two other patients showed gallium uptake in skull metastases of a thyroid carcinoma.

The right lobe of the thyroid gland of patient 2 showed gallium uptake, but the left lobe did not. Gallium does not concentrate in fibrotic or necrotic tissue as was demonstrated by the correlation between the lack of gallium uptake in this left lobe and the histological appearance of the tissue from this area.

Patient 5 had a follicular thyroid carcinoma proved by histological examination after thyroidectomy and a liver metastasis proved by radioactively iodine scanning. A larger accumulation of

Ga-67 than in the normal enclosed liver tissue was expected (5). However the liver scan after administration of Ga-67 showed a locally decreased uptake. The patient had been treated previously with a therapeutic dose of radioactive iodine and tyrosine; therefore the decreased gallium uptake was probably due to fibrotic or necrotic tissue.

Of 6 patients with a negative gallium scan of the thyroid gland 2 had a small thyroid carcinoma. As mentioned by Vaidya et al. (4) the detectable amount of Ga-67 within the tumour probably depends on the volume of tumour tissue and whether the differentiated thyroid carcinoma is more or less a follicular one. The resected thyroid tissue of patients 6 and 7 appeared to contain a small volume of malignant cells. That might be the explanation of the negative gallium scan.

Gallium uptake was seen in follicular adenocarcinoma of the thyroid gland, but it also occurs in leiomyosarcoma, as was shown in patient 8. Gallium uptake in the reticuloendothelial system is well known (1) but it also occurs in lymphocytic thyroiditis, as was shown in patient 9.

The result of the gallium thyroid scanning of a 57-year-old woman with a nodular goitre and a mild hyperthyroidism has not been included in the Table. The thyroid gland did not show Ga-67 uptake. After treatment with a thyreostatic drug the patient became euthyroid.

Scanning with Ga-67 seems to be useful in the preoperative diagnosis of thyroid carcinoma. Ga-67 decays by electron capture ($T_{1/2} = 78$ h), giving γ -emissions only so it cannot be used for therapy.

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RENAL INVOLVEMENT IN ESSENTIAL CRYOGLOBULINAEMIA

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Abstract. A report is presented on five patients, three female and two male, who exhibited cryoglobulinaemia and symptoms of renal involvement. Four of the patients had purpura eruption during some phase of their illness. These five cases may be characterized as essential cryoglobulinaemia, since the conditions associated with secondary cryoglobulinaemia could be ruled out. Glomerular and vascular lesions were demonstrated by light microscopic studies of percutaneous renal biopsy specimens. Immunofluorescent microscopy revealed nodular deposits of immunoglobulin and complement in the glomerular capillary walls. T biopsy specimens were investigated by electron-microscopic means, and in one case subepithelial discrete deposits were demonstrated. These deposits are identical with the "immune observable" in acute post-streptococcal glomerulonephritis. Two patients completely recovered from the renal disease and two died.

The term cryoglobulin was introduced by Lerner and Watson in 1947 (10).

Cryoglobulins is the term applied to denote serum proteins that precipitate and gel spontaneously at low temperatures and return to soluble form on elevation of the temperature. The precipitates may be composed of several immunoglobulins (IgG-IgM, IgG-IgA or IgG-IgA-IgM), or simply consist of one immunoglobulin element (IgG or IgM). Complement components and fibrinogen are also detectable in the precipitates. It seems that IgG-IgM cryoglobulinaemia is the most frequent form of mixed cryoglobulinaemia (2).

Mixed cryoglobulinaemia has been demonstrated in association with many diseases, including streptococcal nephritis (6), connective tissue, malignant-haematological, and viral diseases (16). Large amounts of cryoglobulins are also observable in clinical syndromes associated with such symptoms as Raynaud's phenomenon, purpura,

serositis, vasculitis and renal damage; these cases may be characterized as essential cryoglobulinaemia.

Many authors have described cryoglobulinaemia associated with streptococcal nephritis, lupus nephritis and polyarteritis nodosa nephropathy (6, 7). This report is concerned with renal involvement in cases of essential cryoglobulinaemia, investigated by the application of light-microscopic, immunohistochemical and electron-microscopic techniques.

MATERIAL AND METHODS

The series comprised three patients treated in the Maria Hospital, Helsinki, and two patients in the Fourth Department of Medicine, Helsinki University Central Hospital, during the years 1967-70. The principal clinical data are listed in Table I. Table II illustrates the level of immunoglobulins in the serum and the composition of the cryoprecipitates. Dissolved cryoprecipitates were tested on Ouchterlony plates against polyvalent anti-human and specific antisera for IgG, IgM, IgA and B₂C. If the reaction was positive, quantitative assay as made by the single radial immunodiffusion technique. In its cases the cryoglobulins could be assayed only qualitatively.

Percutaneous renal biopsy was affected by means of Menghini needle with an external diameter 1.4 mm. One piece of the specimen was fixed in 1.5% glutaraldehyde for light-microscopic and electron-microscopic studies. The pieces to be studied electron-microscopically were postfixed in 2% OsO₄ in 0.1M cacodylate buffer and embedded in Epon 812 or Araldite. Ultrathin sections, doublestained with uranylacetate and lead citrate, were studied in Siemens Elmiskop I at 80 kV. The stains used for light microscopy were: haematoxylin-eosin, periodic acid-Schiff, and methenamine silver thiofast, and Congo red for amyloid.

The second piece of the biopsy specimen was placed in physiological NaCl solution and sectioned at 6 microns in cryostat at -20°C. After the sections had been washed

Table I. Main data and clinical course of five patients with essential cryoglobulinaemia

Case no.	Age (yr)	Sex	ESR (mm/h)	Purpura	BP (mmHg)	Serum Cr (mg/l)	GFR (ml/min/1.73 m ²)	Urinary findings	Clinical course
1	73	♀	32	—	120/70	80	97	Haematuria	Recovery
2	53	♂	110	+	140/90	90	—	Haematuria	Recovery
3	25	♀	83	+	180/120	170	—	Proteinuria	Death
						530		Haematuria	
4	71		93	—	180/100	310	26	Haematuria	Death
						510			
5	61	♂	112	—	150/90	110	97	Haematuria	Recovery

in phosphate-buffer-saline solution and fixed in 95% alcohol for 10 min, they were stained with fluorescein-conjugated anti-human globulin and anti- β_2 -C antiserum. The methods applied have been described previously (15).

CASE REPORTS

CASE 1

A 73-year-old pensioned charwoman who had suffered from back pain and arthritis for more than 10 years. During the last few years she had experienced increasing sensitivity to cold and shortness of breath out-of-doors at low temperatures. Since 1960 she had constantly had an elevated ESR, varying from 30–50 mm/h. In 1966 she had been thoroughly examined in the Mäda Hospital by reason of gross haematuria, but the cause remained obscure. In autumn 1969 a purpuric eruption appeared on the lower extremities and microscopic haematuria was detected. The patient was readmitted for evaluation.

The general condition was good. The lower legs were slightly oedematous, and exhibited a purpuric eruption. BP was 120/70. A gentle systolic murmur was heard on auscultation over the heart. All the joints were intact; no signs of arthritis. Laboratory tests: ESR 52 mm/h, Hb 117 g/l, leucocytes $5\,000 \times 10^6/l$, thrombocytes $153 \times 10^6/l$. Normal renal function, creatinine clearance 97 ml/

Microscopic haematuria was repeatedly observed, the urinary sediment contained 3–7 erythrocytes per visual field. No proteinuria or bacteriuria. Normal serum electrolytes. Total serum proteins 62 g/l. Normal electrophoretic distribution of proteins. Serological tests for rheumatoid factors negative. No Le-cells. Antinuclear antibodies of IgM class positive in titre of 1:80. Normal ASO. Direct Coombs reaction and tests for cold agglutinins were

negative. The patient was treated with prednisolone, 30 mg/day initially later 10 mg/day. During this period diminution in haematuria was observed. A second renal biopsy specimen did not display any diminution in haemoglobin deposits as compared with the first specimen. Follow-up investigations have shown continuous microhaematuria.

CASE 2

A 53-year-old metal worker who had been treated for hepatitis at local hospital 20 years previously but had not subsequently shown any signs of liver disease. No family history of rheumatoid disease. In Feb. 1970 the patient had bout of gingivitis and sinusitis and two months later became acutely ill with fever, excess nasal haematuria. On admission to local hospital he was afebrile and had typical purpuric petechiae on the lower extremities and abdomen. ESR 110 mm/h. Later ++ and WaRo L-8. No Le-cells, ASO <80. The patient was treated with prednisolone, 20 mg/daily. In April 1970 he was admitted to the Fourth Department of Medicine, Högskola University Central Hospital.

The patient's general condition was good, BP normal. No purpura, oedema or signs of serious connective tissue disease. ESR 78 mm/h, Hb 158 g/l, leucocytes $9\,100 \times 10^6/l$, thrombocytes $120 \times 10^6/l$. Microscopic haematuria was present, 20–30 erythrocytes per visual field, but no proteinuria. Renal function normal, serum creatinine 90 mg/l. Total serum proteins 100 g/l. Immunoelectrophoresis showed polyclonal increase of IgG globulins. Normal IgA and IgM fractions. The composition of the precipitates and the serum concentration of immunoglobulins are indicated in Table II. Ultracentrifugation of serum proteins demonstrated an increase in 7S globulin. ASO normal. No Le-cells, no antinuclear antibodies. Coombs reaction negative. No cold agglutinins.

The patient was treated in hospital for six weeks, during which time the general condition steadily improved, the haematuria disappeared, and the ESR returned to normal. The patient was discharged without any drug prescription.

CASE 3

A 25-year-old farmer's wife who had repeated respiratory infections at school age. Late in the summer of 1964 her lower legs became oedematous and purpuric eruption appeared on them. As she also displayed joint symptoms,

Table II. Levels of IgG, IgM and IgA and composition of cryoprecipitates in serum of cases 1, 2 and 5

Case no.	Serum (mg/l)			Cryoprecipitates (mg/l)			
	IgG	IgM	IgA	IgG	IgM	IgA	Total
1	13 800	1 320	2 320	250	—	—	250
	62 000	norm.	7 950	580	190	90	820
5	28 700	1 430	4 830	850	—	510	1 360

Table III. Renal biopsy findings

Case no.	Glomeruli	Tubuli	Interstitial tissue	Arteries
1	10 normal, 1-2 hyalineal in 1 segmentary thickening of the capillary wall and possibly focal proliferation	Slight patchy atrophy	Slight focal scarring	Normal
2	1 normal; 1 with pericapsular fibrosis, thickened basement membrane and broadened mesangium	Slight focal atrophy	Diffusely increased slight focal inflammation	In some arterioles hyaline PAS-positive thickening of the wall
3	10 with lobulation, broadened mesangium, cellular proliferation, capillary adhesion	Normal	Diffusely increased no inflammation	In some arterioles intimal thickening and cellular proliferation of the wall
4	5 normal; 5 with focal proliferations, broadened mesangium, necrotic segmentary thickening of basement membrane	Focal atrophy	Focal scarring, inflammation	In some arterioles slight intimal thickening of the wall
5	5 normal; 2 with pericapsular fibrosis, capillary adhesion, possibly focal proliferation	Normal	Diffuse scarring	Normal

recurrent rheumatoid arthritis was suspected and gold treatment was started; this was discontinued after three months by reason of proctitis. In spring 1967 small skin ulcerations and purpura appeared on the legs. The patient was then pregnant. She had normal delivery in the autumn; during her stay in hospital marked cryoglobulinaemia was observed, with cryocrit values of 30%. The patient is anemic and had a markedly elevated ESR. She was recruited to the Fourth Department of Medicine, Helsinki University Central Hospital.

The patient was in poor condition on admission. The ankles were markedly oedematous, the skin was pale, the liver enlarged. BP 190/120 mmHg, pulse frequency 116/min. No purpuric eruptions in the skin. Laboratory tests: Hb 105 g/l, HCR 31 leucocytes $6900 \times 10^9/l$, thrombocytes $344 \times 10^9/l$, ESR 83 mm/h. Serum electrophoresis: total proteins 53 g/l, γ -globulin normal, α_2 -globulin increased. Immunoelectrophoresis: polyclonal increase in IgM, domination in IgG and IgA. Ultracentrifugation of serum demonstrated an increase in macroglobulins at 6th. A test for cryoglobulins was positive, with cryocrit above of 40%. No L_a-cells or antinuclear antibodies were found, and serological tests for rheumatoid factors are negative. Symptoms of malignant hypertension and severe renal involvement were predominant. Serum creatinine rose from its initial level of 170 to 530 mg/l. Constant haematuria and proteinuria. Secondary infection of the urinary tract as occasionally present.

The patient was treated with prednisolone, 20-40 mg/day diuretics and antihypertensives (Nepresol, Aldomet and guanethidine). Later cytotoxic therapy (Imurel 100 mg day) was started and continued for 2 months. During this period the ESR fell from 116 to 45 mm/h

and the cryocrit from 30 to 15%. The level of immunoglobulins did not change. The patient died in April 1968.

Autopsy findings: Normal bone marrow, normal synovial tissue. The loose connective tissue of the heart was increased, with moderate infiltration by histiocytes. The kidneys exhibited signs of acute pyelonephritis along with glomerular and vascular lesions.

Case 4

A 71-year-old unskilled worker with a history of arthritis for several months at the age of 45. These symptoms disappeared and no signs of joint disease had appeared subsequently. Before the patient became acutely ill, he had experienced increased sensitivity to cold, in the form of pain at the extremities and chest at low temperatures out-of-doors. He was admitted to Mäsa Hospital for examination.

On admission the patient was in moderately good condition, but complained of fatigue and dyspnoea. BP 190/100 mmHg, pulse 84/min. A purpuric eruption was present over the ankles. Signs of acute disease. Laboratory tests: ESR 93 mm/h, Hb 109 g/l, leucocytes $6400 \times 10^9/l$. Constant haematuria was present, and later also proteinuria. Bacterial culture as negative. Renal function was unimpaired, serum creatinine 310 mg/l, endogenous creatinine clearance 26 ml/min. I urography demonstrated delayed excretion of contrast medium, but no anatomical changes. Bone marrow puncture showed moderate secondary plasmocytosis but no signs of myeloma. Serum proteins 63 g/l, albumin fraction decreased, γ -globulins somewhat increased. Immunoelectrophoresis was within normal limits. Repeated tests for cryoglobulins were positive. The components of the precipitates were not



Fig 1 Case 1. Lobulation of the capillary tuft and cellular proliferation. Haematoxylin and eosin stain. 450



Fig 2 Case 3. Lobulation of the capillary tuft, capsular adhesions and segmentary thickening of the basement membrane. Periodic acid silver methenamine. 450.

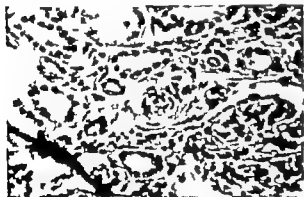


Fig 3 Case 3. Lamellar thickening and cellular proliferation of the arteriolar wall. Haematoxylin and eosin stain; 280.

quantitatively determined. ASO normal. No Le-cells antinuclear antibodies.

The predominant clinical feature was steady deterioration in the general condition and the rapid progression of renal disease. The patient was treated with prednisolone, initially 50 mg/day later 30 mg/day. During steroid

therapy an initial reduction in serum creatinine to 250 mg/l occurred, but this effect was transient. The patient died in May 1969 of acute pulmonary oedema following blood transfusion.

Autopsy findings: Moderate coronary atherosclerosis, small areas of infarction in the myocardium, chronic endocarditis. A large area of infarction in the spleen. Chronic glomerular and vascular changes in the kidneys.

Case 5

A 61-year-old male park worker, who had frequently misused alcohol during a period of 20 years, was admitted to Maria Hospital in June 1970 by reason of poor general condition, fever and bronchitis. Ten years previously he had had a hospital examination by reason of epigastric symptoms, but no gastric ulcer or biliary disease was observable. In 1966 the patient was admitted to a neurological department as a consequence of vertigo and difficulties in walking, but the cause remained obscure. Before the present admission to hospital the patient had spent several days out-of-doors in cold weather and had been misusing alcohol for a lengthy period.

On admission the patient was in poor condition and the lower legs were oedematous. There were no petechiae, BP was normal. Laboratory tests: ESR 132 mm/h, Hb 93 g/l, leucocytes $15\,800\ 10^9/l$, thrombocytes $472\ 10^9/l$. Bone marrow puncture was normal. Serological tests for rheumatoid factors were negative. Normal ASO titres. Repeated urinalyses demonstrated constant haematuria, but no proteinuria. Renal function was assessed serum creatinine 110 mg/l, endogenous creatinine clearance 97 ml/min. Normal I. urography. Immunoelectrophoresis: polyclonal increase of IgG and IgA, IgM only moderately increased. A liver biopsy specimen was normal. The patient was treated with prednisolone in decreasing doses. He was discharged in satisfactory condition. The microhaematuria disappeared.

HISTOPATHOLOGICAL AND IMMUNO-HISTOCHEMICAL RESULTS

All the renal biopsy specimens were studied by light microscopy. The lesions observed are indicated in Table III.

In no case did the kidney specimen display a completely normal structure. Two specimens exhibited impressive glomerular changes, with necrosis, proliferation broadening of the mesangium, and thickening of the basement membrane (Figs 1-2). In three cases the changes were slight and of a stationary character: pericapsular fibrosis or complete hyalinization and capsular adhesions. In these cases the proliferative changes were slight or doubtful.

The tubuli showed slight focal atrophy or normal structures. The interstitial tissue exhibited either diffuse or local scarring, with or without

varying degrees of inflammation. The blood vessels were mostly normal. In some arterioli the arterial wall was thickened and contained hyaline and PAS-positive deposits. In others the thickening was lamellar with increase in the cellular elements of the media and intima (Fig. 3).

Biopsy material from cases 1, 4 and 5 was examined by means of immunofluorescent techniques. Nodular deposits of immunoglobulin, and complement along the basement membrane in the glomeruli, were observed in every case (Fig. 4). The interstitium and tubuli were free from deposits.

Electron-microscopic studies

Glomeruli from case 5 exhibited large discrete nodular deposits on the epithelial aspect of the capillary basement membrane (Figs 5, 6, 7). These deposits were of the type normally termed "hump" by kidney pathologists (8, 14). In addition to typical humps, many deposits of the same texture were embedded in the basement membrane (Fig. 8). The basement membrane proper displayed various degrees of thickening at many points.

The endothelial and mesangial cells exhibited local proliferation and hypertrophy with a concomitant increase in the mesangial matrix, resulting in local condensation of the capillary tuft and narrowing of the capillary lumen (Fig. 5).

Morphologically the subepithelial deposits were the same as the "humps" described by many authors in acute post-streptococcal glomerulonephritis (8, 13, 14). The deposits also resembled those reported in experimental serum sickness nephritis (1).

No kind of deposit, or any other of the glomerular changes mentioned above, were discernible in the kidney biopsy specimen of patient 1.

DISCUSSION

In each of the present cases symptoms of renal involvement, such as proteinuria and haematuria, were the predominant clinical feature. Three patients were acutely ill, while in the remainder the course was chronic. One patient had an elevated ASO titre during the acute stage, but no other evidence was discernible, such as acute tonsillitis or positive pharyngeal bacterial culture, indicating acute post-streptococcal glomerulonephritis.

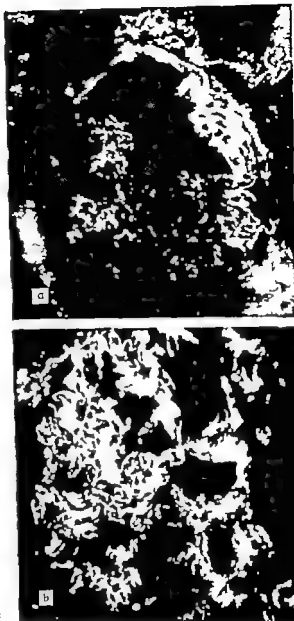


Fig. 4. Case 5. Nodular deposition of immunoglobulin (a) and B₂C (b) along the glomerular basement membrane in kidney biopsy specimen stained with fluorescein-labelled anti-human immunoglobulins and anti-B₂C antibodies respectively.

In the other four cases acute post-streptococcal glomerulonephritis could be ruled out. Signs of acute connective tissue disease were also lacking. Anti-nuclear antibodies, of IgM class, were discovered in one patient, and tests for rheumatoid factors were positive in another. In previous studies rheumatoid factor activity has been dem-



Fig. 5 A part of a glomerulus showing accentuated lobulation and partly occluded capillary lumina as the result of endothelial (mes) and mesangial (mesr) cell hyperplasia and increased mesangial matrix (mesr). In the lower right corner is a large subepithelial deposit (→) which is shown in larger magnification in Fig. 7. $\times 800$.

onstrated in patients with nephritis and cryoglobulinaemia (12).

Renal involvement has been demonstrated in patients with either essential or secondary cryoglobulinaemia (2) and the renal damage has been attributed to immunological causes.

Theoretically the renal lesions may have two different pathogenetic mechanisms they may be caused either by circulating non-glomerular antigen-antibody complexes precipitating in the glomerular capillaries, or by antibodies against the glomerular basement membrane. Convincing evi-

dence of these kinds of mechanism has been produced in animal experiments. The first mentioned type is discernible in Dixon's chronic immune-complex nephritis, in which a proportion of the animals develop typical proliferative glomerulonephritis after repeated intraperitoneal injections of serum protein. In glomeruli have revealed globulin and complement deposits. In microscopic investigation of the glomeruli, the following changes have been observed: hypercellularity, hyperplasia of the epithelial cells, and thickening of the basement membrane.

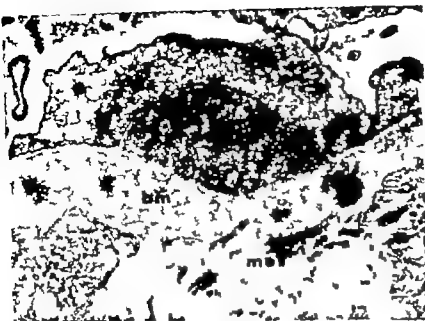


Fig. 6. A subepithelial deposit (dep) adjacent to mesangial region (mes). The deposit is separated from the lamina densa of the basement membrane (bm) by narrow light zone. 20 300.



Fig. 7



Fig. 8

Fig. 7. Larger magnification of the subepithelial deposit shown in Fig. 5. The deposit (dep) is darker and somewhat more granular than the basement membrane (bm). 14 400.

Fig. 8. Ovoid extramembranous deposit (dep). The basement membrane (bm) is locally thickened, probably as a result of the deposit. 14 400.

served in human pathological studies that SLE nephritis arises from endogenous immune complexes (9) while bacterial antigen-antibody complexes are considered to be a contributory cause of renal involvement in bacterial endocarditis (3). The renal damage in experimental serum sickness has a similar pathogenesis (5).

In renal disease induced by basement membrane antibodies, such as Goodpasture's syndrome the

pathology is different. Electron microscopy reveals thickening of the inner portion of the basement membrane with subendothelial granular densities, and immunofluorescent microscopy mainly shows a fine linear fluorescence along the basement membrane (4).

Melzer and Franklin (12) have detected diffuse, chronic glomerulonephritis in three patients with essential cryoglobulinaemia, the glomerular depos-

its consisted of IgG and IgM. In acute cases of glomerulonephritis, deposits consisting mainly of IgG or IgG-B₂C components have been encountered in the basement membrane (6).

The subepithelial deposits observed by electron microscopy and the deposits demonstrated in the present cases by immunofluorescent microscopy suggest that the renal damage is attributable to circulating immune complexes. The electron-microscopic findings displayed a striking resemblance to those reported in experimental serum sickness and Dixon's chronic immune complex nephritis. However it is impossible to draw any definite conclusions in regard to the pathogenetic mechanism or development of the renal lesions in our study. It is also impossible to decide with any certainty whether the immunoglobulin deposits in the glomeruli were identical with the serum cryoglobulins in the individual cases. The resemblance between the electron-microscopic finding, and that reported in acute post-streptococcal glomerulonephritis is not surprising, as it is believed that circulating immune complexes also play a significant part in streptococcal glomerulonephritis.

All of the present patients received steroid treatment. One patient (no 3) was also given cytostatics, which led to a marked diminution in cryoglobulins and depression of the ESR. However no abatement of the symptoms became apparent. Mathison et al. (11) recently described a patient with IgM IgG cryoglobulinaemia reacting favourably to cyclophosphamide in conjunction with splenectomy. Reports of steroids having a favourable effect are relatively few

and evaluation of the results of steroid therapy in the present study is difficult, as two of the patients displayed a tendency to spontaneous remission.

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PREGNANCY IN A PATIENT AFTER CADAVERIC RENAL TRANSPLANTATION

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Abstract. This paper describes the course of pregnancy in a 35-year-old primipara after transplantation of a donor kidney. Signs of chronic rejection occurred during this pregnancy and increased sharply after about 35 weeks. A cesarean section was performed, after which the graft function did not improve. Data on histocompatibility of donor husband, patient and child, and other possible relationships between pregnancy and graft function are discussed. The urinary total estrogen excretion was low during pregnancy. This could not be ascribed to abnormalities in placental steroid synthesis. The child is a boy in good physical and mental health.

In a survey of pregnancies after renal transplantation Golby (5) reported on pregnancies in 19 women, 18 of whom gave birth to a healthy child.

To the best of our knowledge only 8 pregnancies after renal transplantation are documented in detail (1, 3, 4, 7, 9, 10, 11, 13). This paper reports on another pregnancy after cadaveric kidney transplantation and presents data on some clinical and immunological aspects.

CASE REPORT

A 35-year-old woman was admitted in June 1965 with terminal renal failure due to chronic nephritis. She was treated by intermittent hemodialysis for three years. Renal transplantation was performed in May 1968 (the donor being a 20-year-old man who had died during an operation performed in extracorporeal circulation). A biopsy taken within one hour after transplantation revealed normal histological picture, without any interstitial infiltrate. The postoperative course was characterized by an oliguric phase lasting 13 days. Acute rejection occurred on day 7 and was controlled by increasing the prednisone dosage. The endogenous creatinine clearance rose to 100 ml/min. In view of developing hypertension, trans-plant arteriography was carried out which disclosed stenosis

of the arterial anastomosis. This was surgically corrected, whereupon the BP returned to normal. The data on this period are presented in Fig. 1.

The patient developed anorexia during treatment hemodialysis, but regained normal metabolism after wards. In 1969 she expressed the wish to become pregnant.

As that time the graft was functioning well, and the BP was normal. Table 1 presents the laboratory studies of the donor recipient and husband as they could be established early in 1969. We concluded that husband and donor had no specificity to react with the recipient was lacking. It therefore seemed unlikely that pregnancy might evoke immunologic rejection on the basis of HLA-incompatibility. Fig. 1 shows the biopsy specimen. Since the transplantation some months a fibrosis had developed. An immunofluorescence study with polymerized streptococci showed left glomerular fluorescence. Operative examination of the biopsy is related to illustrations.

In view of these findings the patient was allowed to discontinue oral corticosteroids. She became pregnant, but had miscarriage having occurred on June 20, 1969. In the second week of pregnancy she was hospitalized with anorexia, bronchopneumonia and weight loss. She made a rapid recovery under antibiotic treatment.

Fig. 1 shows the functioning of the transplant during pregnancy. The endogenous creatinine clearance was 60-80 ml/min—a level lower than that prior to pregnancy and postpartum.

Proteinuria gradually developed to a level of 1.5 g/day. Immunosuppressive medication was continued at daily dosage of 1.5 mg prednisolone and 150 mg azathioprine. The urinary total estrogen excretion remained subnormal (Fig. 3). After the 34th week fetal growth was believed to have ceased; urinary protein excretion had increased. The biochemical data during this period are presented in Fig. 4. Within a few days proteinuria increased to about 12 g/day. The possibility of graft rejection was considered. A primary cesarean section was performed after 35 weeks gestation. A healthy son was delivered (weight 3300 g, height 43 cm). There were no neonatal anomalies. During and after delivery metabolic immunosuppressive medication was increased to 12 mg/day in the event of rejection (15). Urinary

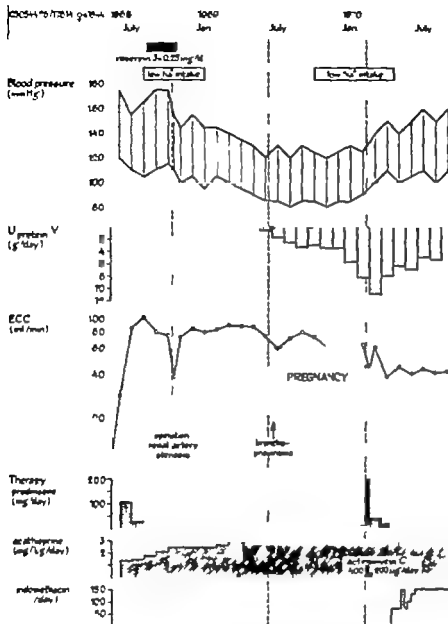


Fig. 1 Clinical and biochemical data after kidney transplantation. U protein = protein concentration of the urine, V = urine volume, ECC = endogenous creatinine clearance.

tion remained high (Fig. 4). The proteinuria during the 48 hours following surgery can in part be ascribed to spinal flow. The endogenous creatinine clearance decreased to about 40 ml/min. A salt-poor diet was prescribed after the operation. Sodium excretion diminished, BP rose to about 160/105 mmHg. The patient's general condition was good.

DISCUSSION

Diminished graft function and proteinuria were principal features during and after this pregnancy. They may be imputed theoretically to three factors: pregnancy as such, the infectious disease in

the second week of pregnancy and immunological aggression induced by the fetus.

The irreversible diminution of graft function immediately before as well as after the cesarean section suggests a relationship with pregnancy. On the other hand the proteinuria developed at a very early stage of pregnancy without a concomitant rise in BP or any other sign of toxemia. The infection in the second week of pregnancy could have also damaged the transplant. This seems improbable, however because there were no symptoms of irreversible transplant affection in this period. In view of an eventual immuno-

Table I. Tissue antigenic properties of the donor, recipient and husband, determined by leukoagglutination in 1969

	HL-A system										ABO system
	4	4b	6	6b	7a	7	7b	7	7d	8	
Donor	+	+	+	+	-	-	+	-	-	-	O
Recipient	+	+	+	+	-	-	+	-	-	-	A
Husband	+	+	+	+	-	-	+	+	-	-	A

logical cause the leucocyte antigens were redetermined in husband, mother and child by the cytotoxic method. Material from the donor was not available. Table II shows that mother and child were "full house"-identical. This seems to exclude induction of rejection by the fetus, at least on the basis of HL-A incompatibility.

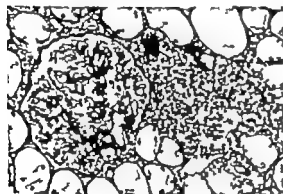


Fig. 2. Transplant biopsy performed in June 1969. Original magnification 246. Silver methenamine stain.

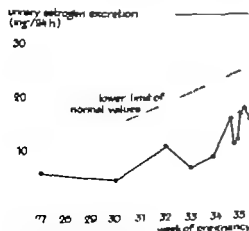


Fig. 3. Urinary total estrogen excretion during pregnancy measured according to van Kessel et al. (8).

We realize that, in view of the new typing results, HL-A incompatibility between mother and child could have occurred. The chance that the child would have inherited specificity 1 from the father was 50%. The donor could have had the specificity 12, because he was 7b positive. The incidence of specificity 12 is supposed to be 29.5% in a random population and 23% in a population of 7b positives (6). Therefore the statistical risk of the presence of a specificity in the child, lacking in the mother and present on the graft, was about 15%.

The influence of pregnancy upon graft function may be reconstructed also in a different way. There is evidence to support the hypothesis that pregnancy is a privileged immunological state (11, 12). One might interpret Fig. 1 in such a way that an already present chronic rejection has slowed down during pregnancy and regained its original strength toward its end.

In accordance with other studies (14) prednisolone and azathioprine medication had not affected the fetus. The child—now almost three years old—has developed normally. In July 1973 the mother's general condition was very good. The renal function was stabilized, endogenous creatinine clearance being about 45 ml/min, DP 130/85 mmHg. Proteinuria (during pregnancy 12 g/24 hours) decreased to 0.6 g/4 hours. Therefore we still think that pregnancy caused the diminution of graft function.

We have no satisfactory explanation for the low estrogen excretion. In the absence of data on plasma estrogen levels it is difficult to establish the role of diminished renal function. Histological examination of the placenta revealed no abnormalities. In vitro incubation experiments were made with placental tissue homogenates. The conversion of tritium-labelled pregnenolone, of ^3C -labelled DHA to estrone and estradiol, and of labelled 16 α -OH-DHA to estriol, occurred at

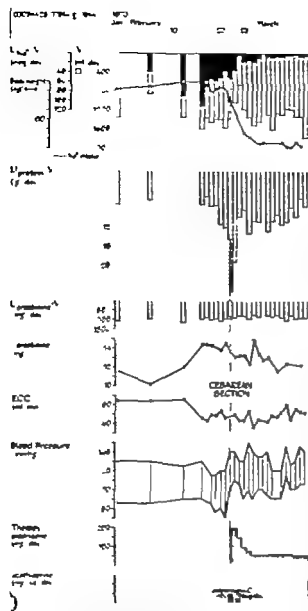


Fig. 4 Clinical and biological data during the weeks before and after cesarean section. P_{crea} = plasma creatinine concentration. Other abbreviations as in Fig. 1

a normal rate. There are, therefore, no arguments to ascribe the low estrogen excretion to a disturbance of steroid synthesis. It seems more likely that the estrogens were low because the fetal adrenal glands were suppressed by prednisolone (2).

Table II. Tissue antigenic properties of the father, mother and child, determined by the cytotoxic method in 1971

	HL-A system	
	7-antiblocus	LA-antiblocus
Father	12, 7	2, 3
Mother	5, 7	2, 3
Child	5, 7	2, 3

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EDITORIAL

PHARMACOGENETICS

When we see a new disease that does not seem to have been described before, our first question should be: Is this man-made, iatrogenic? The cause is often a new pharmaceutical preparation that may have very specialized side-effects. One of the most peculiar among these is the extrapyramidal motor effect of prochlorperazine on some patients but not on all. It is clear that not only the toxicity of the drug but also the susceptibility of the patient are responsible for the symptoms. This is illustrated very clearly by the development of severe parkinsonism in some patients, who have received an ordinary normal dose of succinyl choline. Further studies have disclosed an inherited sensitivity also in many family members of such patients. It has been demonstrated that the intoxication is caused by a decreased ability to break down the toxic substance and this is explained by a lack in or defective function of definite enzyme, an esterase.

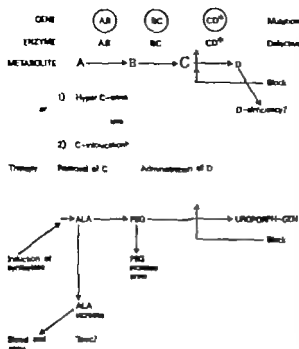
The conditions is thus one among the many inborn errors of metabolism that are all caused by a defect in some enzymatic system or in the malformation of some other active protein. The classical inborn error of metabolism is caused by a metabolic block at some definite point in the metabolic sequence of chain of substances but the clinical effects may be mediated by the accumulation of toxic substances, either endogenous or exogenous, as in the example I have just mentioned. These patients suffer from what I have called biochemical malformations.

There has been an increasing number of such conditions described in the medical literature and the events are different even if the underlying principle of metabolic block is always the same. Perhaps the most widely known and also the most common among these idiosyncrasies against certain drugs is hemolytic anemia after primaquine, sulpho preparations and many other synthetic chemicals. The reason for this condition is of sudden because of great practical importance was the last war American troops were treated prophylactically against malaria with primaquine on grand scale. Among these soldiers there are many coloured people and in this group there occurred a very large number of cases of acute hemolytic anemia. The condition had already been described in 1922. It was later established that all these persons and many of their relatives had deficient function of an erythrocytic enzyme, G6PD. This is involved in the breakdown of glucose in the red cell. If this process is defective, there is an accumulation of H_2O_2 and this is thought to damage the red cell in such a way that it is more

easily destroyed. The result is hemolytic anemia. These variations are much more common in certain ethnic groups and rare among Northern Europeans.

A number of idiopathic conditions with hyperhemolysis became clear when G6PD was analyzed in the carriers. It was then found that there was definite liability to hemolysis in certain families. In some families the disease is caused by the ingestion of fava beans (Vicia faba). Such favism had been known for a very long time in Italy. Another acute hemolytic disease is the so-called Malaria spring fever. It had long been known that sulpho drugs may cause acute hemolytic anemia in few persons. It is now clear that disturbances in the formation of G6PD molecules are the cause of all these troubles and a large number of different mutations have been described. As a matter of fact no less than ± 100 different mutated G6PD molecules have been observed. Practically all of them have subnormal capacity as catalysts of glucose metabolism but interestingly enough one (G6PD Hektoen) is more active than the usual ("normal") G6PD. This is probably the only instance, where a mutation has been shown to produce more effective product than the one that has survived through natural selection!

In order to get an idea about the many types of genetic influences on the handling of drugs by the patient's body I shall only quote a few more examples. Isoniazid (INH) was found to give severe neurological symptoms with polymyocystopathy in many patients. An excellent study was performed on a very large group of normal persons in order to see how quickly they are able to break down the drug (2). It was then found that the distribution curve of the elimination time showed very marked bimodality with nearly equal division. As a matter of fact slow (S) and rapid (R) inactivators were just as common. In such cases it is impossible to speak about normal or abnormal and it is not the question of a rare inborn error of metabolism even if the analysis of heredity showed the presence of two genes S and R that obeyed the Mendelian laws with absolute precision. It is also interesting that the investigation was planned in such way (analysis at 6 hours) that the differences came out beautifully. If 2 hours had been chosen, normal curves would have been found. The same persons, who are slow regarding INH, are also slow in inactivating other drugs such as sulphadiazine proving that the same enzyme system is at work on many different drug molecules (in this instance acetyltransferase).



Also for drug interaction pharmacogenetic analysis is an important instrument. Diphenylhydantoin has very marked capacity to "improve" its own breakdown after repeated administration. This is a very interesting phenomenon, namely enzyme induction by the substrate. It is obviously very similar to the formation of a specific antibody after challenge with an antigen. We have reason to assume that this type of depression of protein forming templates occurs more widely than in immunological connections. It has been observed that this enzyme induction by diphenylhydantoin is severely inhibited by the simultaneous administration of INH and still the two drugs are broken down by different enzymes. Thus it cannot be the question of simple competition for the enzyme.

Other inherited disturbances of drug metabolism and thus of drug action are operative in the families with resistance to cocaine therapy or to succinylcholine because they have supernormal enzyme activity. In this group of resistant persons the very rare metabolic defect called the Leach Nyblom syndrome occupies a unique position. The afflicted persons have a very severe neurological disturbance with self-mutilation. Their enzyme output is such that they cannot break down porphyrins to uric acid. Therefore they cannot metabolize 6-mercaptopyurine, thiopental or succinylcholine and are completely resistant to these extremely toxic cytostatic drugs because breakdown product of the drug is the active compound. The affected sites in these families a deadly poison is completely innocuous.

The biologic half-life of phenylbutazone is 3 hours in the rabbit and 70 hours in man. This accounts for the fact that the rabbits need much higher dosage to obtain equal plasma levels and the same levels are necessary for the drug activity in both instances. Also between individuals the differences may be very big. An intramuscular

dose of 800 mg phenylbutazone may have a biological half-life of 1.5 day in one individual and 6 days in another. The same is true of diphenylhydantoin.

The importance of genetic factors is investigated in the paper by Hoch Andreasen et al. in *Acta Medica*, June 1973 (p. 361). This group of Danish investigators treat the problem of genetically controlled drug interaction. They have studied the factors that influence the breakdown of diphenylhydantoin in the body and find that it is inhibited by phenylbutazone. The study of identical and paternal twin pairs leads them to the idea that this is genetically determined. They assume that the two drugs compete regarding the microsomal hydroxylation system in the liver. If their ideas are genetically correct, levels of this system would be the explanation of the differences in sensitivity.

Another paper (in this number) treats the interesting problem why only a small minority of alcoholics develop porphyria cutanea tarda (PCT) and still alcohol must be an important etiologic factor. One of the authors (O. L.) has already published studies in this journal indicating that heredity may play a part in the development of this disease (3). Also other authors (5) have indicated that there are scattered observations of familial occurrence. In this paper on p. 465 the authors analyze Lundvall's very large material from a genetic point of view. The finding that cells from fine needle biopsies of the liver show strong fluorescence in the porphyrias and also in some of their clinically healthy siblings probably indicates that they are latent porphyrias, do carry the trait but do not become manifest until they consume alcohol in large quantities or are exposed to other toxic influences. This is another example of "pharmacogenetic" problem even if most people could not regard alcohol as a pharmaceutical preparation! We do not know how it should regard the mechanism of action but it is a fact that the liver is the chief organ involved both in the metabolism of alcohol and also of some porphyrias. Lundvall has previously produced convincing evidence that liver iron is of paramount importance for the production of PCT (3). The mechanism is obviously complex. It seems clear to me that conditions leading to hemochromatosis (e.g. acquired hyperhemolytic syndromes) may have the same effect as alcohol on persons with latent PCT. Riholm has shown that there is a reciprocal relation between porphyria in bile and urine. If he believes that these porphyrias are initially excreted in the bile and are therefore more or less "hidden" in the liver. If bile excretion is impaired (alcohol?) then the porphyria in the circulation increases and causes light sensitivity before they are excreted in the urine. There are thus still many problems to solve but the present paper by Lundvall and his group is a contribution on the way.

The most classical example of pharmacogenetically determined disease is another porphyria as a matter of fact II as the first in which inherited sensitivity to certain drugs gave metabolic upsurge and it may well be said that it was the first in this important group of diseases. I mean acute intermittent porphyria (AIP). In several papers from 1936, 1939 and later we pointed out that this was an inborn error of metabolism but the site of the metabolic block could not be located (4).

Certain facts such as the occurrence of increased porphobilanogen—but not of ALA—in the urine from asymptomatic children, who might be carriers of the trait indicated that the block was immediately after PBG.

The idea about a metabolic block became less interesting when it was found that the drugs causing severe attacks such as phenobarbital also induced the formation of ALA in increased amounts. The idea about enzyme induction via ALA synthetase dominated the discussion for long time until Marver and collaborators were able to demonstrate that the enzyme that converts uroporphyrinogen—a colourless early stage of porphyrin synthesis—to the red uroporphyrin is defective in AIP. There was thus both block of the chemical kind in known errors but the role of the drugs in this pharmacogenetic disease was played through enzyme induction. Even if we know about its stages in the process we still have to ask: how is the villain in the drama, is there one toxic metabolite that causes all the dramatic and often deadly symptoms with severe colic, constipation, purpura and general neurological disturbances? There are many indications that similar symptoms occur in other conditions with increase in ALA such as some other types of porphyria and above all in lead poisoning.

Let us try to draw a schematic picture of the processes in standard inborn error of metabolism (see Fig.) and compare it with the hypothetical mechanism in AIP.

It is interesting that the mortality of acute porphyria—

disease that can hardly be successfully treated once the attack has started—has been reduced dramatically in Sweden ever since a paper written in 1939 demonstrating the dangers of synthetic drugs above all barbiturates and the necessity to recognize the disease before it is too late (4). During the decade 1927–36 55% of patients in our big family died from porphyria, in 1937–46 25% and in 1947–56 only 4%. The number of new carriers discovered in Sweden was constant, 75–85 in each period.

This seems to be another example beside G6PD and other erythrocytic enzyme deficiencies showing the importance of pharmacogenetic outlook.

As Post Script it may be added that the last number of *The Annals of Internal Medicine* contains a paper by Cecil Watson demonstrating feedback effect of haem on ALA synthetase proved by excellent effect on patient in an attack of AIP.

Jan G. Waldenström

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BOOK REVIEW

Neuromuscular blocking and stimulating agents, volumes I and II Edited by J. Cheymol, 654 pp. £17.50. Pergamon Press, Oxford, England 1972.

The neuromuscular junction is a classical model for studies of the physiology and the pharmacology of synaptic transmission. Particularly in recent years the use of electrophysiological micro-techniques and of various toxins for the identification and quantification of cholinergic receptors has greatly advanced our knowledge of this synapse. It therefore appears appropriate that the International Encyclopedia of Pharmacology and Therapeutics has published a book entitled "Neuromuscular blocking and stimulating agents".

The book is divided into two volumes and its 71 chapters cover the morphology physiology pharmacology and selected pathophysiological and clinical aspects of the neuromuscular junction. It has been produced under the editorship of Professor J. Cheymol, who has been fortunate in obtaining as authors for the different chapters a number of prominent and experienced scientists.

The emphasis in the book is clearly on basic physiological and biochemical aspects of the neuromuscular junction, and in these fields it contains a number of excellent chapters. I should like to mention particularly Hubbard's well dis-

posed description of the physiology of the normal neuromuscular junction and Bowman and Webb's chapter on acetylcholine and anticholinesterase drugs. Both chapters contain a wealth of up-to-date factual information and, in addition, they review recent ideas and speculations as to the physiological and pharmacological mechanisms involved in the various steps of the transmission process. All chapters are not equally well written, but most of them are of an acceptable standard. Some chapters do not include the more recent advances, but in general this has been amended by the addition of an addendum or a supplementary reference list to the chapter. The clinical part of the book is more fragmentary but I should like to mention a highly authoritative chapter on myasthenia gravis by Osserman, Foldes and Genkins.

In summary the book contains some well written chapters on the physiology and the pharmacology of the neuromuscular junction and is to be recommended to the clinician or for that matter the basic scientist interested in these aspects of the neuromuscular synapse. The book has a valuable subject as well as authors index.

Stephen Thesleff Lund

MASSIVE DIGOXIN INTOXICATION

Report of Two Cases with Pharmacokinetic Correlations

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Abstract. Two patients who had ingested digoxin in great doses with suicidal intent are presented. They reached plasma digoxin levels of 8.1 and 14.4 ng/ml respectively. The urinary excretion, and for one of them the successive loading of tissue digoxin, were followed. Discrepancies were found due to one of the patients being previously digitalized, the other having for many years been on digitalis treatment. During the first days of intoxication atrial arrhythmias and A-V block predominated. No ventricular arrhythmias were seen. At a level less than 2 ng/ml the digitalis-induced dysrhythmias disappeared.

Contrary to a fairly high frequency of iatrogenic digitalis intoxications (8, 12) deliberate massive intoxications do not seem to be common. Some reports, however, are given. Gaultier et al. (7) have presented 62 cases and Pébay-Peyroula et al. (10) another 17 cases of self-poisoning with heart glycosides. In most of these cases digitoxin was ingested, in some lamotokide C. Nono had taken digoxin. In the latter communication the glycoside concentration in plasma and urine was measured by means of atomic absorption spectrophotometry of non radioactive rubidium taken up by erythrocytes. Five patients with suicidal and accidental digoxin ingestion, in whom serum glycoside levels were estimated with a radioimmunoassay were recently reported by Smith and Wilkerson (15).

The present communication reports two cases. The subjects had taken overdoses of digoxin with suicidal intent and were for several days studied with estimates of plasma and urine glycoside concentrations. In one of them the digoxin concentration in skeletal muscle was also analysed. The results are correlated to the clinical findings.

MATERIAL AND METHODS

The two patients were treated at the Intensive Care Unit of the hospital. Besides conventional 12-lead ECGs, 10-min ECG strips were registered several times a day. Blood samples for liver alla, electrolytes and serum creatinine were also drawn daily. Blood for plasma digoxin assay was taken at admission to hospital. Samples were then drawn in the morning at intervals of 24 hours. Urine was collected from admission to the next morning. It was then sampled in 24-hour portions.

The method used for plasma digoxin assay was modification of the ^{86}Rb (rubidium) method, previously described in detail (3). For analysing glycoside concentrations in urine, principally the same method was used. When preparing the standard samples, normal urine, not containing digoxin or other heart glycosides, was used instead of normal plasma. For both plasma and urine assay convenient sample volumes were chosen that did not contain more than 6 ng/ml.

In case 1 small pieces of skeletal muscle were taken on different days from the anterior tibial muscle. After being weighed they were homogenized in Potter-Elvehjem homogenizer in 10 ml saline solution in ice bath. 3 ml of this homogenate was then extracted with 10 ml dichloromethane. After centrifugation the dichloromethane phase was transferred to new tubes and the homogenate was reextracted with another 10 ml organic solvent. From the sampled extract 1 ml were taken for each estimate evaporated to dryness, and redissolved in 1 ml of a methanol-water solution. The procedure then followed that for plasma assay in separate experiments known amounts of digoxin, 14-245 ng/g, were added to homogenate prepared from muscle specimens from non-digitalized subjects; $93.6 \pm 3.5\%$ (mean \pm S.D.) was recovered.

CASE REPORTS

Case 1

Male 67 years old, weight 77 kg; moderate ethanol abuse. He had always been in good health. Two days before admission he was prescribed digoxin, 0.25 mg day for

Table I Laboratory data

Case	Creatin./% (mg/dl)		Creatin. clear (ml/min)		K /% (mEq/l)		Na /% (mEq/l)		Mg /% (mEq/l)	
	1	2	1	2	1	2	1	2	1	2
Day										
1	1.4		49		3.9	6.1	133	140	1.2	
	1.5	1.6	80	40	4.8	5.3	143	140	1.5	
3	1.1	1.3	34		4.5	4.5	152	143		1.8
4	1.0	1.2	53		4.4	4.3	146	138		1.8
5		1.3			4.4	3.9	142	139		1.6
6	0.9	1.9			3.8	3.5	142	134		1.7
7						4.3				
8		1.5					140		1.8	
9		1.5							1.8	
10		1.5		40		4.7	138		1.8	
11		1.5		45		4.4	138			
12		1.5				5.1	139			

atrial fibrillation. On that occasion he also received promethazine as a hypnotic, 25 mg at night. On the following day he was found unconscious in his home with the two medicine bottles empty: each had contained 100 tablets.

On admission to hospital he was unconscious, his skin was ashen and signs of vomiting were registered. BP was 130/70 mmHg. He had an arrhythmia with a heart rate of about 100 beats/min. ECG showed atrial tachycardia with block. Initially he had hypomagnesaemia; other wise normal electrolytes (Table I). Serum creatinase was somewhat elevated.

Plasma digoxin concentration on the first two days was 8.1 ng/ml. After a rather slow decrease the half-life was approximately 36 hours from the 5th day (Fig. 1).

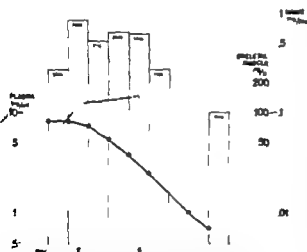


Fig. 1 Case 1 —●— plasma digoxin levels (ng/ml). □—□— concentration in skeletal muscle. The bars represent the 4-hour urinary excretion of digoxin (mg/day). At the top of each bar the 4-hour urine volume (ml) is given.

The urinary excretion of digoxin in the first 4 hours was about 250 µg. During the next 4 days it was at a level of about 700 µg/day; thereafter the excretory amounts successively decreased (Fig. 1). Digoxin concentration in skeletal muscle, estimated on days 1, 2, and 3, showed somewhat increasing tendency at level of about 100 µg/g tissues (Fig. 1).

After an initial period of atrial tachycardia with block and with a heart rate of about 100 beats/min the ventricular rate decreased to 50–60/min. The atrial rate is about 30/min. On the 2nd and 3rd days the rhythm was mainly atrial fibrillation with a ventricular rate of 50–60/min. Frequent periods of A-V nodal rhythm were seen. On the 5th day the same dysrhythmias were still present with ventricular rate of 50–55/min. Then the ventricular rate successively rose to 65/min and the periods of A-V nodal rhythm disappeared. A few ventricular premature beats were registered during the last days; otherwise no ventricular arrhythmia was observed.

During the second day the patient began to regain consciousness. After period of excitement and hallucinations, on the 3rd day he could answer questions. His general condition gradually improved. A therapy potassium was administered intravenously and the acid-base balance was corrected. Because of pneumonia he was given ampicillin and, during the period of exclusion, chlorothalidol (Hembarbitol X).

Case 2

Male, 78 years old, weight 60 kg; rheumatic fever in 1911. In 1958 atrial fibrillation and mitral insufficiency were diagnosed. He has since then been treated with digoxin, 0.25 mg once daily. In Sept. 1971 he developed signs of mental depression. On the day before admission he ingested 7.5 mg digoxin, probably with suicidal intent. He was admitted to the hospital with increasing nausea about

4 hours later. He had probably vomited. On arrival he was conscious but unresponsive. There were no signs of congestive heart failure. BP was 150/80 mmHg, heart rate about 60 beats/min. ECG showed sinus rhythm with 2nd

degrees A-V block and periods of A-V nodal rhythm. Left anterior hemiblock and abnormal QRS complexes in V and V indicated previous myocardial damage. On admission the patient showed signs of dehydration. An initial hypokalaemia was normalized after 2 days. Table 1 gives his electrolytes and renal function tests.

The digoxin concentration in plasma on the first day was 14.4 ng/ml. It decreased during the first two days, with an approximate half-life of 24 hours (Fig. 2). On the following 6 days a somewhat slower decrease was registered ($T_{1/2} \sim 60$ h). On the 6th day his plasma level fell below 2 ng/ml. Urinary excretion of digoxin decreased concurrently with decreasing plasma levels, from about 1 000 $\mu\text{g/day}$ during the first days (Fig. 2). About 2 500 μg are recovered in the urine during the 12 days investigated.

The arrhythmias seen on the ECG on admission are placed on the second day by an atrial flutter with varying A-V block, interrupted by A-V nodal escape beats with long R-R intervals of about 3 sec. Ventricular rate was otherwise about 55-60/min. In between, atrial fibrillation with ventricular rate of 40/min was registered. During the 2nd and 3rd days sinus bradycardia with 1st degree A-V block ($P-Q = 0.33$ sec) was seen, alternately with a supra-ventricular arrhythmia with varying A-V block ($P-Q$ 0.24 and 0.42 sec). Frequently sino-atrial block was observed. Ventricular rate increased during these days from 40 to 60/min. From the 4th day sinus rhythm with frequent atrial and nodal premature beats was registered. The P-Q interval is normalized on the 6th day. Sinus rhythm with moderately frequent supraventricular and few ventricular premature beats remained during the following 6 days.

His general condition during the first days in hospital was rather good considering the circumstances. His senses successively decreased. On the 5th day he became temporarily worse; the systolic BP fell to 80 mmHg, temperature increased to above 40°C , and short period of oliguria developed. These symptoms were explained by urinary infection, which on treatment was rapidly cured. His general condition then improved, except for his symptoms of mental depression.

Except for atropine intravenously no specific anti-arrhythmic drugs were administered during the interdiction period.

DISCUSSION

According to present experience signs of digitalis toxicity often appear at plasma digoxin levels above 2 ng/ml (2, 5, 11, 13, 14). In the two patients investigated in the present study we have registered plasma digoxin values of 8.1 and 14.4 ng/ml, respectively. Out of more than 5 000 plasma samples assayed for digoxin at the laboratory during recent years, values above 7 ng/ml have been registered in only a few patients, all with signs of a severe digitalis intoxication. In three the outcome was lethal.

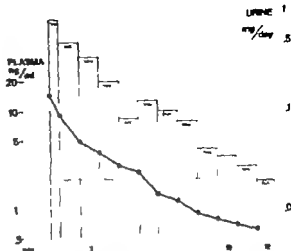


Fig. 2. Case 1. Symbols as in Fig. 1

In the two cases reported here very high plasma levels were found after arrival in hospital, and from the case histories these data agreed with the presumption that they had ingested massive over doses of digoxin. It was believed that case 1 could have taken about .5 mg of the glycoside together with 2 500 mg promethazine. The plasma glycoside values and the total urinary excretion of about 3 mg, however indicated that a great part of the ingested dose had probably not been absorbed. His vomiting most probably explained this.

The two cases are interesting from the pharmacokinetic standpoint, as case 1 might be regarded as previously non-digitalized, whereas case 2 had been treated with digoxin for several years. This is probably the main reason for the divergences observed regarding plasma concentrations and urinary excretion in the two patients (Figs. 1 and 2). It is obvious that the plasma level in case 1 was the same on the first two days after admission. Only a slow decrease was registered on day 3. This could be because he had simultaneously taken promethazine. It is probable that the anticholinergic effects of this drug delayed his gastric emptying and prolonged the time of absorption. Furthermore, the absorbed digoxin was rapidly distributed to the body tissues. In fact, such a distribution could explain why the urinary glycoside excretion was lower on day 1 than on day 2, despite similar and rather large urine volumes. Direct evidence for a continued tissue

loading is given by the results obtained from analyses of muscle biopsies. The glycoside levels registered in these specimens revealed a high concentration in skeletal muscle already on day 1 (~ 70 ng/g). Even higher values were found on days 2 and 5. It is interesting that the last value was registered when the plasma digoxin levels decreased. It seems probable that a peak concentration in the muscle occurred sometimes on day 3 or day 4. The recorded glycoside levels are higher than those obtained in patients treated with a normal maintenance digoxin dose. Bertler et al. (4) recently found a mean value of about 40 ng/g skeletal muscle in such patients. In the heart muscle the corresponding value was about 90 ng/g.

In case 2 the very high initial plasma levels rapidly decreased and with a $T^{1/2}$ of about 24 hours during the first days. The urinary excretion measured during the first day was almost 1 mg, despite a rather small urine volume. The excretion then decreased with decreasing plasma levels. These findings might be due to this patient being well digitalized and having well "loaded" tissues when he ingested the glycoside overdose.

As can be seen in Fig. 2, the plasma level registered in the morning of the 6th day was somewhat higher than expected. But on day 5 he had high fever, low BP and low urine volumes, which probably explain the value. The renal function was more impaired in case 2, reflected in a prolonged elimination of digoxin. From the 3rd to the 8th day an approximate $T^{1/2}$ of 60 hours could be calculated. In case 1 the corresponding value was 36 hours. Doherty (6) calculated the normal $T^{1/2}$ for digoxin at about 32 hours. It has previously been shown by Marcus et al. (9) that an increase of the diuresis might have little effect on the excretion of digoxin. Similar observations were made during the present investigation. It is obvious that the digoxin amounts excreted have little relation to the daily urine volumes.

High doses of digoxin might cause a hyperpotassaemia (11, 15) because of inhibitory effect on cation transport mechanisms. This was observed in case 2, who on admission had a serum potassium value of 6.1 mEq/l. In both patients several rhythm disturbances were registered during the first days of observation. Despite the high plasma digoxin levels no ventricular premature

beats were seen. Recently Smith and Willerson (15) in a similar study reported the same absence of ventricular arrhythmia in three patients who showed no evidence of heart disease. In two patients with coronary artery disease ventricular arrhythmias were seen as an initial manifestation of toxicity. The present study indicates that this discrepancy between patients with and without previous heart disease is not absolute, as in one of the patients studied the ECG showed signs of preexisting myocardial damage.

The abundance of cardiac arrhythmias was notable; these however were not serious for the patients. They gradually diminished and disappeared when the plasma digoxin had reached a level of about 2 ng/ml. Only atrial premature beats, probably not related to toxicity, were seen at lower levels. This is in good agreement with the results in a prospective study on, *inter alia*, toxic patients followed with long ECGs and plasma digoxin estimates for 5 consecutive days (2) and gives further support for the previous statement that the toxic borderline for digoxin is about 2 ng/ml.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (14X 2839), the Swedish National Association against Heart and Lung Diseases, and the Medical Faculty the University of Lund, Sweden.

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D Falch

From the Hormone and Isotope Laboratory Akty Hospital Oslo Norway

Abstract. The influence of renal function, body size and age on the plasma concentration and urinary excretion of digoxin in 53 hospitalized patients on maintenance dose of 0.25 mg has been studied. The mean plasma concentration was 1.01 ng/ml (S.D. 0.43); 64% of the patients were undernourished (plasma values < 1.00 ng/ml). The plasma level was strongly influenced by kidney function, but this was reduced at least 30% (glomerular filtration rate, renal plasma flow), and to a minor degree by age. Studies on the effect of body size on the plasma digoxin level gave equivocal results, but this factor was of minor importance compared to that of kidney function. The urinary excretion of digoxin (on average 33.9% of the administered dose) was significantly reduced as kidney function decreased. Variations in body size did not affect the excretion, nor was an increased urinary output observed during regular maintenance administration of diuretics (furosemide, thiazides).

Valuable knowledge concerning the pharmacokinetics of digoxin in human beings has been obtained in short-term experiments with labelled digoxin (3, 4, 5, 6, 7, 8, 9, 12, 13, 15, 16).

The introduction of a radioimmunoassay of digoxin (18) has made it possible to follow the concentration of the drug in blood and urine and thus obtain information about the pharmacokinetics of the drug in patients given digoxin therapy.

The present study was designed to investigate the influence of renal function, body size and age upon the plasma concentration and urinary excretion of digoxin in hospitalized patients on a daily maintenance dose of the drug (0.25 mg Lanoxin,® Wellcome). The Lanoxin tablets used in this study were manufactured before the recent alteration in the Lanoxin production process. Digoxin determinations were performed by radioimmunoassay (15).

MATERIAL

The 1 males and 11 females of this study were hospitalized in the medical departments of Akty Hospital. The mean age of the female group was 73.6 years and of the male group 70.4 years. Thirty-six of the patients (68%) had coronary heart disease, 8 valvular heart disease, 3 hypertensive heart disease and 4 chronic pulmonary heart disease. Classification of the cardiac function according to the New York Heart Association was difficult because of the high age of the patients. The patients were divided into two groups as follows.

Group I: 34 patients with compensated heart failure on a continuous dose of 0.125 mg digoxin twice a day (8.30 a.m. and 5 p.m.), for at least 2 weeks.

Group II: 13 patients with congestive heart failure who had not previously taken digoxin. They were given 1 mg digoxin orally divided into 4 doses on the first day and 0.75 mg divided into 3 doses on the second day. Later they received the same daily maintenance dose as group I.

Thirty-three patients were on low sodium diet. Twenty-seven were treated with diuretics (furosemide (Lasix,® Hoechst), trichlormethiazide (Furtilan, Schering) or polythiazide (Renase, Pfizer)).

METHODS

Digoxin in plasma and urine was determined by radioimmunoassay (15). The sensitivity of this assay was in the order of 0.25 ng/ml digoxin, the precision within an assay 6.5%, precision between assays 8.7%, and recovery 97.5%. Blood for determination of digoxin was drawn at 8 a.m. before the morning dose of the drug was given. Determination of digoxin in the urine was performed with the same assay after diluting the urine 1:100 in phosphate buffer. In the reference standards normal human plasma was replaced by normal human urine in these instances. When sequential determinations of the digoxin concentration were performed in plasma or urine of patient, the samples were stored at 4°C until all samples were collected, whereafter the determination was made in the same assay series.

Chemical creatinine determinations were made on a

Table I Digoxin in plasma determined on 6 sequential days in 10 patients on a continuous daily dose of 0.25 mg

Pat. no.	Plasma concentration of digoxin (ng/ml)						Mean (ng/ml)	S.D. (ng/ml)	Creatinine clearance (ml/min/1.73 m ²)
1	1.65	1.95	2.10	1.55	1.80	1.75	1.80	11.1	38
2	1.90	1.25	1.50	1.30	2.15	2.20	1.65	25.5	35
3	0.80	0.95	0.95	0.80	1.45	1.15	1.01	24.5	39
4	0.70	0.65	0.80	1.10	1.05	1.00	0.88	21.8	57
5	0.75	0.75	1.00	0.90	0.80	0.80	0.83	12.0	78
6	0.55	0.80	0.80	0.95	0.80	0.85	0.79	16.5	76
7	0.70	0.80	0.80	0.70	0.80	0.95	0.79	11.4	65
8	0.75	0.65	0.75	0.65	0.70	0.70	0.67	13.4	87
9	0.55	0.60	0.75	0.80	0.55	0.65	0.65	15.4	91
10	0.60	0.60	0.75	0.60	0.60	0.55	0.62	11.3	100

Technicon AutoAnalyzer (method N 11). Endogenous creatinine clearance was determined on the basis of 24-hour urine collection, the serum creatinine concentration being measured during the first hour. In group I the creatinine clearance was performed only once in each of 17 patients, and during 3 sequential days in 14 patients. In group II the clearance was performed on the 8th day after the start of digitalization in 7 patients, and in another 7 patients on the 8th, 9th and 10th days. Urine collection was unsuccessful in 8 patients due to incontinence of urine.

Unless otherwise stated, the first-day creatinine clearance also was used in the calculations for patients who had their creatinine clearance determined for 3 sequential days. Each patient is thus only represented once in the calculation and figures involving clearance.

In 38 patients ¹²⁵I orthotolodo-hippurate clearance was done with constant infusion technique. Five determinations had to be excluded because constant plasma level was not obtained with this infusion rate, leaving 33 cases for evaluation.

The technical performance of the radioimmunoassay of digoxin excluded interference from the radioactivity of the ¹²⁵I hippuran when this compound was present in the samples.

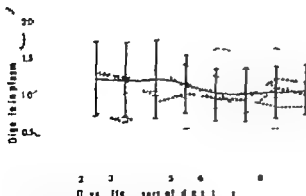


Fig. 1 Plasma concentration of digoxin from the 3rd to the 10th day after start of digitalization in 7 patients. The unbroken horizontal line shows the mean value the vertical line indicates 1 S.D. from the mean.

RESULTS

The mean plasma concentration of digoxin in 53 patients (determined on the 8th day in group II) was 1.01 ng/ml (S.D. 0.42). Thirty-five patients (64.0%) had plasma values ≤ 1.00 ng/ml.

Table I shows the plasma concentration of digoxin during 6 sequential days in 10 patients from group I. The results are arranged according to the mean plasma concentration of each patient and ranged from 0.62 to 1.80 ng/ml. There is a striking inverse relationship between plasma concentration and creatinine clearance: the product of mean plasma concentration of digoxin and creatinine clearance was about 60 in all instances. The plasma concentration varied considerably from day to day in the same patient (11.1–25.5% of the mean, average 16.3%).

In group II the plasma level of digoxin was determined daily from the 3rd to the 10th day after start of digitalization in 7 patients (Fig. 1). The plasma level varied from patient to patient.

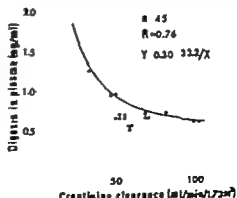


Fig. 2 Correlation of creatinine clearance and plasma level of digoxin.

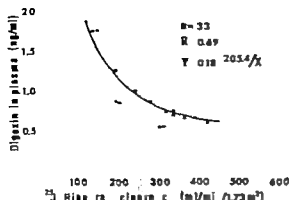


Fig. 3. Correlation of hippuran clearance to plasma digoxin level.

From the 6th to the 9th day the plasma concentration ranged from 1.85 to 0.60 ng/ml, and the individual variation from 20.9 to 3.3% of the mean, average 6.3%.

In a total of 53 patients (groups I and II) creatinine clearance was successfully performed in 45. Mean creatinine clearance in these 45 patients was 57.4 ml/min/1.73 m² (S.D. 22.4). Only 7 patients (13.2%) had a clearance above 80 ml/min/1.73 m². When the group of 45 patients was divided into 2 fractions, those above 70 years and those below 70 years, the mean creatinine clearance for the former was 48 ml/min/1.73 m² (S.E.M. 3.8) and for the latter 70 ml/min/1.73 m² (S.E.M. 4.6). Similarly the hippuran clearance in 15 of the patients below 70 years of age was 320 ml/min/1.73 m² (S.E.M. 22.8) and in 11 of those above 70 years of age the mean clearance was 244 ml/min/1.73 m² (S.E.M. 16.4). As is evident, the difference in the kidney clearance function is significantly different in the two age groups.

Fig. 2 shows the relationship between digoxin

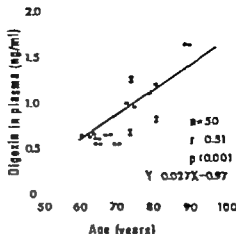


Fig. 4. Correlation of age to plasma digoxin level.

concentration in plasma and creatinine clearance performed on the same day and after stabilization of the plasma digoxin level (on the 8th day in group II). The plasma level of digoxin, related to hippuran clearance, is shown in Fig. 3. Hippuran clearance was performed on the same day as the creatinine clearance in 27 patients. There was a close relationship between creatinine clearance and hippuran clearance ($r=0.77$, $p<0.001$).

The plasma concentration of digoxin was also influenced by age (Fig. 4). Only patients above 55 years of age were included.

In order to study the influence of body size upon the plasma level of digoxin, it was necessary to eliminate the effect of variation in kidney function. Patients with the same creatinine clearance (ml/min) were therefore paired into two groups; 111 pairs were obtained. Differences in digoxin plasma concentration, age, body weight, body length and body surface in the two groups were calculated, using the *t*-test for paired observations (Table II). In the two groups body

Table II. Paired data (mean \pm S.D.) of creatinine clearance for 36 patients

	Creatinine clearance (ml/min)	Digoxin in plasma (ng/ml)	Age (y.)	Body weight (kg)	Body height (m)	B.S.A. (m ²)
Group I	55.9 (23)	0.97 (0.40)	71.8 (6.9)	67.9 (11.6)	1.61 (0.1)	1.74 (0.16)
Group II	55.8 (22.7)	0.96 (0.43)	72.4 (10.5)	59.1 (10.9)	1.61 (0.1)	1.61 (0.14)
<i>t</i>		0.065	0.041	2.947	1.118	2.877
<i>p</i>		>0.5	>0.5	0.01	0.01	0.05

T-test for paired data.

A is used, same.

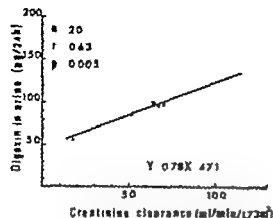


Fig. 5 Correlation between the excreted amount of digoxin in the urine and the creatinine clearance. Mean values from 3 sequential days (72 h).

weight, body length and body surface differed significantly while no difference was found for age and digoxin concentration.

On the other hand, when males and females—with significant differences in body size—were compared, the calculated ratio of plasma concentration of digoxin to body size (m^2) differed significantly ($p < 0.05$) (t -test for unpaired data) in spite of a similar age distribution. For comparative purposes males and females were placed in separate groups, but paired with regard to creatinine clearance: 12 pairs were obtained. The difference in plasma digoxin concentration in the two groups was calculated using the t -test for paired observations. In this instance no difference was found ($p < 0.25$).

The relationship between the excreted amount of digoxin determined in the urine from the patients who collected urine for 3 sequential days and the corresponding creatinine clearance is shown in Fig. 5. The values represent the mean three 4-hour urine collections.

The amount of digoxin excreted in urine (mean $93.2 \mu g/4 h$, S.D. 8.4) was 35.9% (S.D. 13.4) of the administered daily dose. The excreted amount was $97.0 \mu g/4 h$ (S.D. 23.1) in group I and $94.5 \mu g/4 h$ (S.D. 39.0) in group II.

Eleven patients who received regular daily medication with diuretics (furosemide, thiazides) excreted $88.6 \mu g/4 h$ (S.D. 4.4), while 10 patients without diuretics excreted $98 \mu g/4 h$ (S.D. 32.8). The difference was not significant ($p < 0.40$). There was no correlation between the

excreted amount of digoxin and body weight or body surface.

DISCUSSION

The plasma concentration of digoxin varied considerably from patient to patient, and also from day to day in the same patient on a constant daily dose of digoxin (Table 1). The variation is far beyond the variation limits of the digoxin assay as can be seen from the precision and accuracy of this determination. The explanation may be found in differences in absorption, excretion, distribution volume or metabolism. Whatever the reason, the observed variations would suggest caution in the evaluation of single results. This variation in plasma concentration may be associated with a variation in pharmacodynamic effect. Repeated determinations are therefore advisable in patients on a continuous dosage of the drug, in order to obtain a safe evaluation. Even with the observed variation it is evident that "equilibrium" for digoxin in plasma in group II was obtained within 6 days after the start of digitalization.

The body size might be expected to influence the distribution volume of digoxin, and therefore also the plasma concentration. The difference in ratio of plasma digoxin to body size between males and females might indicate an influence. When the patients were compared with regard to creatinine clearance however no influence was found. This is in agreement with the findings of Ewy et al. (11), who gave a single dose of labelled digoxin to obese patients. Similar observations have been made for digitoxin (14, 17). The presented observations indicate that the influence of body size on the plasma concentration of digoxin is outweighed by the kidney clearance function for the drug.

Age was an important factor determining the plasma digoxin concentration (Fig. 4). During the process of aging one may expect a reduction in functional capacity of the organ handling the absorption, metabolism and excretion of digoxin. The creatinine clearance (glomerular filtration rate) and hippuran clearance (renal plasma flow) were significantly reduced with increasing age and a reduction in kidney function is probably most important in this connection.

The presented observations are in agreement

with the findings of Evered and Chapman (10), who studied 22 subjects with evidence of digoxin intoxication and 86 patients without signs of intoxication. The plasma level of digoxin and the age of the subjects with digoxin intoxication were found to be significantly higher than in the non-intoxicated group. A significantly higher blood urea was found in the toxic group than in the non-toxic group in the subjects above 55 years of age. There was no significant difference in sex ratio and body weight between the two groups.

The plasma concentration of the drug in the patients on a daily dose of 0.25 mg was increased considerably when glomerular filtration rate (2) and renal plasma flow were reduced at least 50% (Figs. 2 and 3). With clearance and flow above 50% the plasma concentration was only influenced to a minor degree. Baylis et al. (1) found no correlation between plasma digoxin levels and creatinine clearance in elderly ambulatory patients. Precise dosage and urine collection, however are difficult in elderly ambulatory subjects.

A therapeutic level between 1.00 and 2.00 ng/ml is usually recommended. Figs. 2 and 3 also illustrate that the maintenance dose of 0.25 mg digoxin used is too small to reach the intended level when the renal plasma flow or glomerular filtration rate exceed 50% of the normal.

As could be expected, there was a close relationship between the renal plasma flow and the glomerular filtration rate.

Some interesting observations regarding the excretion or metabolism of digoxin can be made from the acquired data. From direct measurement in the urine it can be seen that the amount of digoxin falls significantly when the creatinine clearance is reduced (Fig. 5). Calculation on the basis of the amounts of digoxin presented to the kidney (the product of digoxin concentration and renal plasma flow) gives reductions of the same order of magnitude as measured in the urine. These calculations may indicate a reduced intestinal absorption and increased intestinal excretion or changed metabolism of digoxin when kidney function is reduced.

The urinary excretion of digoxin was similar in the patients given a constant medication of furosemide or thiazide, and in those receiving no such treatment. The acute initial effect of the

administration of a diuretic on the urinary digoxin excretion is at present under study. The effects of the diuretics are of clinical importance. When renal plasma flow in a patient on a continuous dosage of digoxin is reduced because of cardiac insufficiency the plasma level of the drug should be expected to increase according to the observations presented (Figs. 2 and 3). A further increase might be expected if furosemide or thiazides are given, because the total body water volume will be reduced when the diuresis increases, and digoxin intoxication might result if the digoxin is not excreted in increased quantities with the augmented diuresis.

ACKNOWLEDGEMENT

This study was in part supported by the Norwegian Council on Cardiovascular Diseases.

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SERUM DIGOXIN VALUES FOLLOWING A DOSAGE REGIMEN BASED ON BODY WEIGHT SEX, AGE AND RENAL FUNCTION

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Abstract. Serum digoxin has been determined by radioimmunochemical method in 99 patients treated with digoxin. Digoxin was administered using dosage regimen with daily maintenance dose equivalent to $(14 + \text{creatinine clearance}/5)$ per cent of loading dose. Three loading doses were used: 0.01 mg/kg b.wt., 0.015 mg/kg and 0.02 mg/kg, respectively. Creatinine clearance was calculated from rapid bedside evaluation based on nomogram. This nomogram requires knowledge merely of the age of the patient as well as sex, b.wt. and serum creatinine. The serum digoxin mean doses are 1.0 ± 0.3 ng/ml, 1.1 ± 0.5 ng/ml and 1.4 ± 0.6 ng/ml in the three groups of patients with maintenance doses corresponding to the three loading doses. The frequency of digoxin intoxication in the three groups was found to be 0, 4 and 8%. The dosage regimen described seems to be a valuable aid to digoxin therapy.

In a recent prospective clinical study of digitalis intoxication the incidence of cardiac toxicity in digoxin-treated patients was found to be 25% (1). In the toxic group of patients the renal function was considerably decreased compared to non-toxic patients, and the mortality was more than twice as high in the toxic group. Another recent study describes a similar high frequency of digoxin intoxication (7). Digoxin is excreted primarily in unaltered form by the kidneys, and with decreasing glomerular filtration rate digoxin half-life in blood is prolonged (5, 9).

Evaluation of the renal function seems to be essential in determining the dosage of digoxin, and Jelliffe has suggested a dosage regimen in which the daily maintenance dose should be equivalent to $(14 + \text{creatinine clearance}/5)$ per cent of loading dose (10, 11). This formula does not seem to be widely used, probably because of the time needed to estimate creatinine clearance. We

have recently described a rapid bedside evaluation of creatinine clearance based on a study of urinary creatinine/kg b.wt. in different age groups. Creatinine clearance can be calculated from a nomogram which requires a knowledge merely of the age of the patient as well as sex, b.wt. and serum creatinine (12, 13).

In the present study serum digoxin values have been estimated following a dosage regimen based on the formula of Jelliffe and calculated creatinine clearance.

MATERIAL AND METHODS

The material comprised 99 patients, 48 females and 51 males aged 45-90 years (average 70). B.wt. varied between 40 and 118 kg (average 65). All patients were normotensive and serum creatinine varied between 0.6 and 3.6 mg/100 ml. The calculated creatinine clearance varied between 15 and 190 ml/min. The patients were divided into three groups: 14 patients received maintenance dose of digoxin corresponding to loading dose of 0.01 mg/kg b.wt., 48 maintenance dose corresponding to loading dose of 0.015 mg/kg and 39 maintenance dose corresponding to loading dose of 0.02 mg/kg. The daily maintenance dose of digoxin was calculated from the formula of Jelliffe using the three loading doses and creatinine clearance evaluated from the nomogram of Kampmann et al. (12). In addition 19 patients admitted to hospital with digoxin intoxication were studied. These patients had all received higher doses of digoxin than the above mentioned groups.

Serum digoxin concentration was measured after at least two weeks of daily stable oral digoxin dose which varied between 0.125 and 1.00 mg. In most of the patients the digoxin value used was mean value of 4 consecutive analyses of serum samples collected at 14-day intervals. The majority of the patients received digoxin preparations from Danmarks Apotekerforenings Kontrol-laboratorer some, however were treated with digoxin Wellcome, 62.5 µg.

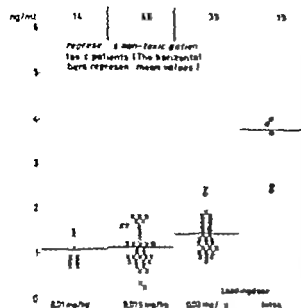


Fig. 1 Serum digoxin in 118 patients with and without toxicity

The ECGs were analysed by one of the authors who had no knowledge of the serum digoxin values. Criteria for digoxin intoxication were based on ECG disturbances occurring in close association with digoxin therapy and disappearing after withdrawal of the drug as described by Smith and Haber (15). Extracardiac manifestations such as nausea, vomiting and diarrhoea are not taken into account (1).

Blood samples for determination of serum digoxin concentrations were collected at 8 a.m. 1–4 hours after the last dose of digoxin. Serum was stored in a deep-freeze refrigerator until the analysis was carried out. Serum digoxin was determined by radioimmunoassay using a modification of the coated charcoal technique of Smith et al. (14) originally used for separation of free from antibody-bound digoxin. The major modification has been the application of a gel centrifugation procedure for separation of free from antibody-bound digoxin (4).

The sensitivity of the method was found to be 0.25 ng/ml. The method was found to give identical values to those of the ^{125}I -Rb method (7).

RESULTS

The results are shown in Fig. 1. The mean value of serum digoxin from 14 patients receiving a maintenance dose corresponding to a loading dose of 0.01 mg/kg b.wt. was 1.0 ± 0.3 ng/ml (\pm SD). In this group no patients showed signs of digoxin intoxication. Forty-six patients received daily a dose corresponding to a loading dose of 0.015 mg/kg b.wt. The mean value for this group was 1.1 ± 0.5 ng/ml. Two of these patients were in-

toxicated. The ECG disturbances in the patient with serum digoxin of 1.2 ng/ml were ventricular frequency of 40/min and ventricular extrasystoles, and in the patient with a value of 2.4 ng/ml first degree A-V block and ventricular bigeminy. In the third group with a digoxin dose calculated from a loading dose of 0.02 mg/kg b.wt. the mean value was found to be 1.4 ± 0.6 ng/ml and differed significantly from the mean values of the two previous groups ($p < 0.01$). Three of these patients showed digoxin intoxication with ventricular frequency of 44/min and 48/min, and one patient also second degree A-V block and ventricular extrasystoles. Serum digoxin values from these patients were 2.3, 1.5 and 2.8 ng/ml, respectively. The last column in Fig. 1 shows serum digoxin values from 19 patients with obvious ECG signs of intoxication. These patients were not included in the prospective study and the majority were examined shortly after admission to hospital. Serum digoxin values from the patients in the prospective group were found to be without significant correlation to b.wt., renal function or to daily digoxin dose.

DISCUSSION

The radioimmunoassay of serum digoxin in the present study using a Sephadex separation technique has previously been shown to correlate well with that of other methods and laboratories (4). It has been generally accepted that patients with serum digoxin concentrations less than 2 ng/ml are unlikely to exhibit signs of toxicity, whereas levels higher than 3 ng/ml almost invariably are associated with toxicity (3, 6, 15). In the present study three loading doses were used, and a maintenance dose corresponding to a loading dose of 0.015 mg/kg b.wt. gave a serum digoxin concentration within the therapeutic range and with a low incidence of toxicity (4%). However, considerable variations in serum digoxin concentrations in all three groups were noticed. This probably reflects a sum of biological variables in the absorption, renal excretion and extrarenal degradation rate of digoxin besides the inaccuracy of the calculation of creatinine clearance and the variation in fat free b.wt. (8). A possible difference in the bioavailability of the two digoxin brands used (IDaA and Wellcome) was evaluated by administration of both products

to four patients. No difference in serum digoxin concentration could be demonstrated, but different bioavailability from the same manufacturer cannot be ruled out.

We find that the dosage regimen described is a valuable aid to digoxin therapy. However it is also demonstrated that digoxin intoxication occurred with serum digoxin values within the accepted therapeutic range, which indicates that factors other than serum digoxin may contribute to the development of digoxin intoxication.

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ABNORMALITIES IN LIVER FUNCTION TESTS DURING LONG TERM DIPHENYHYDANTOIN THERAPY IN EPILEPTIC OUT PATIENTS

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Abstract. Fifty-one epileptic out-patients on long-term anticonvulsant treatment with diphenylhydantoin (DPH) alone or combined regimens have been investigated and compared with a group of 250 control patients. The epileptics had significantly raised serum alkaline phosphatases, serum alkaline aminotransferase, and plasma prothrombin time. Serum albumin was significantly decreased, as well as serum calcium and serum bilirubin. In male epileptics significantly raised serum phosphate concentrations were found. There was no significant correlation between the clearance rate of DPH and the liver tests. It is suggested that the inducing effect of DPH and other anticonvulsant drugs may explain the above findings in liver function tests and calcium metabolism. The possibility remains, however, that prolonged administration of these drugs to humans may eventually result in liver damage.

Finkelstein and Arleff (3) were the first to report findings of raised serum alkaline phosphatases in patients treated with diphenylhydantoin (DPH). In recent studies increased serum alkaline phosphatase activity has been found in 29 (17) and 60% (7) of DPH-treated patients. Richens and Rowe (17) also showed that in 47 epileptic patients with raised serum alkaline phosphatase 28 had an increased activity of the liver fraction and 18 a rise of the bone isoenzyme. A decrease in serum calcium was found in 36 (22.5%) of their patients. Serum albumin and serum alkaline aminotransferase activity were normal.

A disturbance of calcium metabolism due to deficiency of vitamin D was suggested. DPH and other anticonvulsants are thought to accelerate the inactivation of vitamin D₃ in the hepatic microsomes as a consequence of enzyme induction (5, 17).

This study was initiated because we observed raised serum alkaline aminotransferase activity in epileptics treated with DPH. Consequently a more detailed hepatological investigation was undertaken.

MATERIAL

Fifty-one epileptic out-patients, 15-72 years of age, subjected to regular follow-up studies, were investigated. Twenty-seven patients were treated with DPH alone for 6 months to 10 years (mean 4.4 years), and 24 with DPH in combination with phenobarbital, primidone, carbamazepine and various psychotropic drugs for 6 months to 23 years (mean 8.5 years). Twenty-five of the epileptics were males, and 76% of the epileptics were below 40 years of age. The control group consisted of 149 males and 111 females from the ENT and Eye Out-patient Clinic with only minor complaints (e.g. protruding ears and abnormalities of refraction). Subjects in the same age groups as the patients were selected. None received any medication or oral contraceptives.

METHODS

Determinations were made of serum bilirubin (14), serum alkaline aminotransferase (9), serum alkaline phosphatase (11), serum calcium (atomic absorption spectrophotometry), serum phosphate (18), serum albumin (12), plasma prothrombin time (15) and serum DPH (19) by the methods indicated.

Plasma clearance of diphenylhydantoin

Knowing the maintenance dose and the serum concentration of DPH, it is possible to calculate the individual clearance rates of DPH at steady state from the equation:

$$CL_{DPH} = \frac{D}{C_{DPH}}$$

Table 1 Serum components in DPH-treated epileptics and in controls

	Epileptics		Controls		Level of significance	
	Mean	95% confidence limits	Mean	95% confidence limits	t-test	F-test
Males						
Bilirubin ^a (μmol/l)	5.6	2.7-11.2	9.8	4.6-18.1	<i>p</i> < 0.001	1
Alanine aminotransferase (U/l)	3	0-43	14	0-38	—	<i>p</i> < 0.01
Alkaline phosphatase (U/l)	46	0-93	35	9-61	—	<i>p</i> 0.01
Phosphate (mmol/l)	1.19	0.75-1.63	1.01	0.65-1.37	<i>p</i> < 0.001	0.1
Albumin (g/mol/l)	600	503-697	656	519-793	—	<i>p</i> < 0.05
Calcium (mmol/l)						
Group A	2.42	2.26-2.58	2.48	2.30-2.66	<i>p</i> < 0.005	0.1
Group B ^b	2.36	2.18-2.53			<i>p</i> < 0.0005	1
Females						
Bilirubin ^a (μmol/l)	4.6	2.5-8.5	6.9	3.5-11.3	<i>p</i> < 0.001	0.1
Alanine aminotransferase (U/l)	18	0-36	12	0-4	—	<i>p</i> 0.05
Alkaline phosphatase (U/l)	37	6-70	29	11-4	<i>p</i> < 0.02	0.1
Phosphate (mmol/l)	1.18	0.82-1.54	1.14	0.80-1.48	0.1	0.1
Albumin (g/mol/l)	581	476-686	635	530-740	<i>p</i> 0.001	0.1
Calcium (mmol/l)						
Group A	2.39	2.17-2.61	2.42	2.20-2.64	0.1	1
Group B ^b	2.30	2.10-2.50			<i>p</i> < 0.0005	0.1

^a Log normal distribution.^b Treated with DPH alone.

Treated with DPH and other drugs.

where *D* is the daily dose of DPH and \bar{C}_{DPH} is the steady state serum concentration. Blood for the DPH analysis was drawn 3-5 hours after the ingestion of the morning dose. The daily dose of DPH varied from 200 to 450 mg.

Statistical methods

Results are expressed as the mean \pm S.D. Student's *t*-tests were performed unless a *F*-test showed a significant unequal variance (*p* < 0.05). The serum bilirubin showed log normal distribution, so the statistical calculations were based on the logarithmic data, and the stated mean values for serum bilirubin are geometric means.

RESULTS

In the control group a significant sex difference in concentrations was found with regard to the following determinations: serum bilirubin, serum alkaline phosphatases and serum albumin. It was therefore necessary to consider each sex separately. No significant differences in the values were demonstrated in patients treated with DPH alone and patients treated with DPH in combination with other drugs (except serum calcium, see below).

The results from the treated patients and the control group are compared in Table 1. Figs. 1 and 2 show the values for the anticonvulsant

treated groups plotted against the range for the control group (95% confidence limits).

Both sexes in the epileptic group demonstrated a significant decrease in serum bilirubin. Also statistically significant differences were found in serum alanine aminotransferase and serum alkaline phosphatases. Twenty-five per cent of the patients had alanine aminotransferase and 22% serum alkaline phosphatases above the normal range (Figs. 1 and 2).

Regarding serum calcium significantly decreased values were found in male epileptics. In the females only patients treated with DPH in combination with other drugs showed significantly decreased serum calcium values. Serum phosphate was significantly increased in male epileptics as a group and 70% of the males had values above the normal range.

The serum albumin concentration was also statistically decreased in the epileptics, and in female epileptics treated with DPH and other drugs a correlation between serum calcium and serum albumin was found (*r* = +0.541 *p* < 0.05).

The prothrombin time was not estimated in the present control group but in a group of 36 normal subjects (mean age 31 years) it was found

to be $89\% \pm 34$ (mean ± 2 S.D.). In the epileptics it was $105\% \pm 60$ (mean \pm S.D.) with a significantly higher variance (*F*-test, $p < 0.01$).

No significant correlation between the activity of serum alkaline phosphatases and serum alanine aminotransferase was demonstrated.

The clearance of DPH in the epileptics treated with DPH alone was 1.3 ± 1.0 l/hour (mean ± 1 S.D.). Neither the level of serum alkaline phosphatases nor that of serum alanine aminotransferase was correlated to the rate of clearance of DPH. In 4 patients toxic DPH levels were observed (i.e. more than 25 mg/l).

DISCUSSION

An explanation of the rises in serum alanine aminotransferase, serum alkaline phosphatases and plasma prothrombin time and of decreased serum bilirubin could be the known inducing effect of DPH and other anticonvulsant drugs. A decrease in serum bilirubin has been shown to occur after the administration of phenobarbital and other inducing agents and has been attributed to an induction of the hepatic bilirubin UDP-glucuronyltransferase (4). In animals phenobarbital increases liver size and hepatic content of enzymes not only of microsomal, but also of cytosolic and mitochondrial origin (8, 16). Phenobarbital increases alanine aminotransferase activity in the liver but not in the serum of mice (8). However in dogs phenobarbital administration raises both the alkaline phosphatases in serum and in liver biopsies (10).



Fig. 1 Serum components in 25 DPH-treated male epileptics and in male controls (mean ± 2 S.D.)



Fig. 2 Serum components in 26 DPH-treated female epileptics and in female controls (mean ± 2 S.D.).

Experimental data suggest that individuals on long-term phenobarbital therapy have an accelerated conversion of vitamin D₃ to more polar metabolites. This may be caused by induction of microsomal enzymes in the liver (5). The low serum calcium levels have been explained by a deficiency of vitamin D and, in fact, osteomalacic bone changes have been demonstrated in adults on long-term anticonvulsant therapy (2). However the raised serum phosphate values in male epileptics are not readily compatible with a deficiency of vitamin D.

DPH has a marked tissue proliferative effect, and hypertrophy of the gums, gross enlargement of lips and nose and general thickening of subcutaneous tissue of face and scalp have been associated with long-term anticonvulsant therapy (10).

The possibility exists that the abnormalities in liver tests and calcium metabolism are due to a functional abnormality in the liver. In animals prolonged administration of some inducing agents may eventually result in metabolic and morphological abnormalities similar to those seen in human liver diseases (6).

Several cases of severe liver damage following DPH therapy have been reported. This reaction is rare, unpredictable, and apparently not related to the dose or concentration of the drug. It is often accompanied by allergic phenomena and develops 2–7 weeks after the beginning of DPH medication. Of 11 such patients compiled from the literature 11 died (13). The liver biopsies

showed liver cell necrosis and often intrahepatic cholestasis.

Further studies, including liver biopsy and quantitative liver function tests, are required to exclude the existence of liver damage in patients on long-term DPH therapy.

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PORPHYRIA CUTANEA TARDA—A GENETIC DISEASE?

A Biochemical and Fluorescence Microscopical Study in Four Families

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Abstract. The siblings of patients with clinically manifest porphyria cutanea tarda (PCT) have been investigated by chemical analysis of urinary and faecal porphyrin excretion and by fluorescence microscopical examination of smears from fine needle biopsy specimens of the liver. Four families and one alcoholic and one non-alcoholic control group were studied. In two families red porphyrin fluorescence was demonstrated in 5 out of 15 asymptomatic siblings, and one sibling who refused liver biopsy was found to have clinically manifest PCT. In the two other families no porphyrin abnormality could be demonstrated in the 7 asymptomatic siblings. In the control group comprising 36 non-alcoholic subjects no porphyrin fluorescence could be demonstrated, but in the alcoholic control group of 39 subjects liver porphyrin fluorescence was found in two. Alcoholic abuse was common in the two families in which several members had porphyrin abnormalities indicating latent PCT. In the group of alcoholics liver porphyrin fluorescence was uncommon. Hence the present results indicate that PCT might be genetic disease.

In most porphyrias there is an evident genetic background. Porphyria cutanea tarda (PCT) is, however usually considered to be a symptomatic disease and is often named symptomatic cutaneous porphyria or acquired cutaneous porphyria. Contrary to the other porphyrias this disease generally manifests itself in middle or late life. PCT is characterized by skin fragility and blisters on skin areas exposed to the sun. Hypertrichosis and hyperpigmentation are common. Biochemically there is gross uroporphyrinuria, while urinary excretion of coproporphyrin is usually slightly increased. In the typical case the excretion of porphyrin precursors (δ -amino-levulinic acid, ALA, and porphobilinogen, PBG) is normal. The most important causative factor is usually considered to be abuse of alcohol. Although PCT has thus

been considered a non-hereditary disorder by most authors and a family history of the disease has been lacking in most series, there have been some reports of a familial occurrence of PCT. Thus Waldenström and Haeger Aronsen (15) described a pair of uniovular twins with the disease and two other families in which more than one member showed signs of disturbed porphyrin metabolism. Single instances of such kindreds have been reported also by others (3, 11, 13, 14, 16).

Waldenström (14) suggested that PCT might be a hereditary disease and that chemical analysis of excreta is an unreliable tool in unrevealing the PCT trait as analysis of excreta in PCT relatives usually gave normal results.

The liver in clinically manifest PCT contains large amounts of preformed uroporphyrin giving rise to a characteristic red fluorescence in ultraviolet light. As reported elsewhere (5), smears of fine needle liver aspiration biopsy specimens can be used directly for fluorescence microscopy. We found that red autofluorescence was present also in smears from patients with clinically latent disease who had normal excretion of porphyrins. Therefore the study of liver biopsy smears by fluorescence microscopy might be a better method than the currently used biochemical methods for revealing a genetic trait.

The main aim of the present investigation was to study the possibility of hereditary mechanism in PCT. This was done by fluorescence microscopy of fine needle liver biopsy smears in siblings of PCT patients. Urinary and faecal porphyrins were also determined.

If PCT is a purely symptomatic disease alcohol

Table I Normal faecal porphyrin excretion ($\mu\text{g/g dry wt}$) according to the method of Holst et al (4)

Author	Population	()	CP			PP		
			Mean	S.D.	Range	Mean	S.D.	Range
Eales and Saunders (1962)	South African (white)	44	7		0-77	28		0-99
Hager Arosen (1962)	Swedish	50				35		
Present material	Swedish	48	5	3	1-11	16	10	4-52
Rimington et al. (1963)	English	21	7	4	0-14	4	11	1-43

being the outstanding causative factor this porphyrin should not be uncommon in alcoholics. Hence a group of chronic alcoholics was also studied. Finally a non-alcoholic control group was included as well.

MATERIAL

Four patients with clinically manifest PCT had several sibs willing to submit themselves to examination by liver biopsy (sibs A, B, C and D). Their parents were not alive and children were not studied. In one of these sibs (B) brother of the proband had a history of skin fragility and blisters on the dorsae of the hands. In the other three PCT probands there was no family history of PCT. Liver biopsy and porphyrin analysis could be performed in all members of three sibships, but in one (B) a complete study could not be performed. In the latter family liver biopsy was performed in 7 of 11 sibs. Porphyrin analysis was performed in all, however. The estimated alcohol consumption in the four families is shown in Tables II-V.

Fifty-nine chronic alcoholics admitted to a mental hospital (Chale II, Luthagen's Hospital, Göteborg) for

acute and chronic alcoholism, and 36 adults without alcoholic abuse, served as controls and underwent a fine needle liver biopsy. The patients with chronic alcoholism all had well documented alcoholic abuse for 10-20 years, many of them even more. No member of the two control groups had a family history of PCT nor showed such symptoms.

METHODS

The fine needle biopsy technique was performed as described by Söderström (1). Long needles etc. used, at least 10 cm in length. The sections were allowed to dry and were examined without previous fixation. Smears put in liver cells were not accepted. The smears were examined immediately for the biopsy using a Zeiss fluorescence microscope with an Osram HBO high pressure mercury burner equipped with Schott BG 1 primary filter as light source. The fluorescent light was filtered through Zeiss 50 barrier filter transmitting above 500 nm. A dark field condenser was used. The fluorescence was graded on an arbitrary scale from 0 to 4 as described earlier (1).

Urinary excretion of ALA and PBG was analysed according to Mauzerall and Granick (7). Faecal coproporphyrin (CP) and protoporphyrin (PP) were determined according to Holst et al (4). This method has been described

Table II Family A (A.4 - the proband)

	Patient					
	A.1 ♂	A.2 ♀	A.3	A.4 ♂	A.5 ♂	A.6 ♂
Born (y)	1903	1905	1907	1910	1919	1923
Studied (y)	1969	1969	1969	1968	1969	1969
Red hepatic fluorescence (grade)		Neg.	Neg.	++	Neg	
ALA (mg/d)	2.5	0.3	2.6	0.5	3.3	2.2
PBG (mg/d)	1.8	0.8	1.2	0.6	1.2	0
Urinary UP ($\mu\text{g/d}$)	130	8	0	3,300	6	0
Urinary CP ($\mu\text{g/d}$)	15*	129	111	130	151	118
Faecal CP ($\mu\text{g/g dry wt}$)	11	4	2	103	5	
Faecal PP ($\mu\text{g/g dry wt}$)	23	10	6	74	5	12
Alcohol consumption ^a						
Last year	Mod.	Insign.	Mod.	Insign.	Mod.	Mod.
Earlier	Large	Insign.	Mod.	Heavy	Mod.	Mod.

^a Heavy (>6 l/mo.), large (3-6 l/mo.), moderate (1-3 l/mo.), small (0.4-1 l/mo.), minimal (0.1-0.3 l/mo.), insignificant (<0.1 l/mo.). The amount consumed was calculated as 40 () ethyl alcohol.

Table III. *Family B (B.7—the proband)*

	Patient										
	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B 10	B 11
	♂	♀	♂	♂	♀	♂	♀	♀	♂	♂	♂
Born (y.)	1912	1913	1918	1920	1921	1922	1925	1926	1927	1928	1935
Studied (y.)	1968	1969	1969	1969	1969	1969	1965	1969	1969	1969	1969
Red hepatic fluorescence (grade)		Neg.		Neg.	+	+		+	Neg.		Neg.
ALA (mg/dl.)	4.0	1.2	3.8	2.6	0.9	3.2	4.2	1.2	4.5	1.9	5.8
PBG (mg/dl.)	2.0	0.5	1.1	1.1	0.6	0.8	1.2	1.6	2.3	0.9	0
Urinary UP (μg/d)	4600	0	0	0	4	32	3500	0	0	0	0
Urinary CP (μg/dl.)	450	110	97	215	63	161	460	86	191	169	97
Faecal CP (μg/g dry wt.)	70	5	2	37	13	5	80	6	21	0	3
Faecal FP (μg/g dry wt.)	106	22	6	78	8	12	73	10	95	13	6
Alcohol consumption ^a											
Last year	Heavy	Insign.	Mod.	Mod.	Small	Mod.	Small	Insign.	Mod.	Mod.	Large
Earlier	Heavy	Insign.	Mod.	Mod.	Small	Mod.	Small	Insign.	Mod.	Mod.	Large

Classification, see Table II.

accepted for clinical purposes. There are, however, some differences concerning stool-porphyrin levels reported in normals. Table I compares the values obtained in normals in the present study with other large control series reported in the literature.

Urinary excretion of uroporphyrins (UP) and CP was determined according to the method elaborated by Dresel et al. (2) as described by Rimington (9). For UP the upper limit of normal variation is usually reported to be 30 μg/24 h, which is largely in accordance with the present results, in normals mean value of 4 (S.D. 7) with range of 0–28 μg/24 h was obtained. The chosen normal upper limit for urinary CP excretion was 200 μg/24 h.

RESULTS

In family A (Table II) there were two siblings, A. 1 and A. 6, who showed red autofluorescence

in the liver biopsy smear. Both had somewhat dark-pigmented skin, but showed no other symptoms of PCT. The one with the strongest autofluorescence, A. 1, also had increased urinary UP excretion and a slightly increased excretion of faecal CP. He had earlier had a large alcoholic consumption. The other brother, who also showed red fluorescence A. 6, had normal porphyrin excretion. The other siblings did not show any PCT symptoms and all had normal porphyrin excretion.

In family II (Table III) manifest PCT was present in one brother B. 1 of the female proband. He had gross uroporphyrinuria and refused liver biopsy. Three other siblings, one brother and two sisters, B. 5, B. 6 and B. 8, showed a

Table IV. *Family C (C.1—the proband)*

	Patient					
	C 1	C 2	C 3	C 4	C 5	C 6
	♂	♂	♀	♂	♀	♀
Born (y.)	1907	1909	1911	1912	1913	1914
Studied (y.)	1967	1969	1969	1969	1969	1970
Red hepatic fluorescence (grade)	+++	Neg.	Neg.	Neg.	Neg.	Neg.
ALA (mg/dl.)	1.1	4.4	2.3	2.5	3.4	4.2
PBG (mg/dl.)	0	1.2	0.6	0.7	0.7	1.7
Urinary UP (μg/dl.)	6360	0	0	0	0	0
Urinary CP (μg/dl.)	280	129	78	177	87	99
Faecal CP (μg/g dry wt.)	70	2	1	1	2	5
Faecal FP (μg/g dry wt.)	11	9	3	2	6	17
Alcohol consumption ^a						
Last year	Insign.	Mod.	Insign.	Heavy	Insign.	Insign.
Earlier	Heavy	Mod.	Insign.	Heavy	Insign.	Insign.

Classification, see Table II.

Table V Family D (D 4—the proband)

	Patient			
	D 1 ♀	D 2 ♂	D 3 ♂	D 4 ♂
Born (y.)	1893	1896	1900	1902
Stoched (y.)	1969	1969	1969	1968
Red hepatic fluorescence (grade)	Neg.	Neg.	Neg.	++
ALA (mg/d.)	1.3	1.0	2.3	0
PBG (mg/d.)	1.0	1.0	1.3	1.3
Urinary UP (µg/d.)	8	2	0	5800
Urinary CP (µg/d.)	68	60	147	240
Faecal CP (µg/s dry wt.)	1	2	7	40
Faecal PP (µg/s dry wt.)	9	12	13	17
Alcohol consumption ^a				
Last year	Insign.	Small	Mod.	Mod.
Earlier	Insign.	Mod.	Mod.	Heavy

Classification, see Table II.

weak red autofluorescence. They had essentially normal porphyrin excretion however one of them (B 6) had a slight increase in urinary porphyrin excretion and another (B 5) a slight increase in faecal porphyrin excretion. Two other siblings, who were asymptomatic, refused liver biopsy. Two of the siblings who did not show red fluorescence B 4 and B 9 had elevated excretions of faecal CP and PP. Sibling B 4 also had elevated excretion of urinary CP.

In family C (Table IV) no sibling showed red fluorescence in the liver biopsy smear. Porphyrin excretion was normal and there were no PCT symptoms.

In family D (Table V) no sibling showed any symptoms of PCT nor any red autofluorescence in the liver and the urinary and faecal excretion of porphyrins was normal.

The excretion of ALA and PBG was normal in all siblings in the four families.

The results of fluorescence microscopy on fine needle aspiration biopsies from the PCT siblings and two control groups are shown in Fig. 1. In the non-alcoholic control group comprising 36 adults, none showed red fluorescence, while in the alcoholic control group comprising 59 subjects, two showed red fluorescence. In one of them urinary porphyrin excretion was slightly increased, while the other showed no such abnormality. None of the alcoholics had ever had skin symptoms. Nor in another series of 70 chronic alcoholics, were there skin symptoms or a history suggestive of PCT.

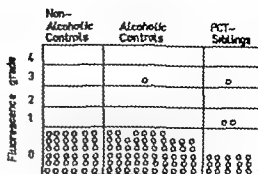


Fig. 1 Results of fluorescence microscopy of smears from fine needle aspiration liver biopsies.

Statistical analysis by the χ^2 test shows that liver fluorescence was significantly more common in asymptomatic PCT siblings than in alcoholic controls ($\chi^2=4.6$, d.f. = 1, $p < 0.05$) and non-alcoholic controls ($\chi^2=7.0$, d.f. = 1, $p < 0.01$).

DISCUSSION

In an earlier study red fluorescence was a consistent finding in smears from patients with PCT of varying clinical activity (5). Porphyrin fluorescence was present also in smears from patients with clinically latent disease who had normal excretion of porphyrins. In the present series of 36 non-alcoholic controls red autofluorescence was not demonstrated in any. In the alcoholics porphyrin fluorescence was demonstrated in 2 of 59 subjects. One of them had a slightly increased porphyrin excretion. None of the alcoholics had symptoms suggestive of PCT. Nor in another 70 chronic alcoholics, were there skin symptoms or a history suggestive of PCT. These results show that fluorescence microscopy of liver biopsy smears is a sensitive screening procedure for PCT. Although, according to most authors, alcohol plays an important causative role in PCT the disorder was not common in alcoholics, which indicates that alcohol perhaps elicits the disease only in individuals with a genetic trait or in whom other porphyrin-eliciting factors may be operative.

The present study of four families showed that more than one member of two families was affected by the disease. In one sibship hepatic porphyrin fluorescence was present in two of four asymptomatic siblings. In another sibship one brother was found to have manifest disease with

gross porphyrinuria, and three other siblings who were asymptomatic were found to have hepatic porphyrin fluorescence. In two others there were slight increases of faecal or urinary porphyrin excretions, the significance of which is difficult to interpret in the absence of liver porphyrin fluorescence. They were both moderate consumers of alcohol.

These results lend support to the suggestion that PCT might be a hereditary disease. The high frequency of the PCT disorder in the present families may be due to exogenous factors. It is noteworthy that in the two families in which several members had porphyrin abnormalities most had a considerable alcohol consumption (Tables II and III), while in the two other families (Tables IV and V) alcohol consumption was smaller. However as noted, porphyrin fluorescence was uncommon in the alcoholic control group.

It is also possible that other factors than alcohol might have caused familial porphyrin disturbances. The members of family A, however were scattered all over Gothenburg and had not lived together since childhood. In family B one brother with manifest disease (B 1) lived in the same apartment as his sister B 5 who was found to have a hepatic porphyrin fluorescence. The rest of the sibs lived in different parts of Gothenburg. It cannot be excluded that the siblings may have been exposed to porphyrogenic substances in childhood with a persistently disturbed porphyrin metabolism.

The most plausible explanation of the familial occurrence of the present porphyric disturbance seems to be a genetic trait. But if PCT is a hereditary disease, the trait must have a low penetrance. Of 52 patients with manifest PCT studied by the authors, a family history was present in a few only. Two patients were uniovular twins earlier reported by Waldenström and Häger Arosen (15). Two pairs of patients were brothers and sisters (including the proband B 7 and B 1 of the present sibship B). Furthermore one male patient had a father who died before the present study and had had a history of blisters of the dorsae of the hands.

A history of PCT in more than one generation is very uncommon. Nürnberg (8) reported a family in which the disease affected three generations. This family was, however incompletely

studied, faecal CP excretion was not determined and probably the disease was porphyria variegata.

Thus, according to available data, PCT might be a hereditary disease in which the trait has a low penetrance or often exhibits a mild expressivity. Whether it is possible to reveal all, or most, carriers by the fluorescence method remains to be shown. It seems, however to be a valuable aid in studies of this kind.

However probably PCT may occur as a merely acquired disease. This is suggested by the out break of hepatic cutaneous porphyria in Turkey probably caused by the ingestion of hexachlorobenzene (10). This porphyria generally occurred in children and adolescents but otherwise showed a great resemblance to PCT. Similarly Bleiberg et al. (1) found an increased UP excretion in 11 of 29 persons working in a factory manufacturing dichloro- and trichlorophenols and three of them had manifest porphyria of PCT type.

Thus, in our opinion, PCT is probably a merely acquired disease but may in most cases have a genetic background, the trait having a low penetrance or mild expressivity and many factors are of importance in eliciting the clinically manifest disease, the most important being the presence of liver iron as shown in earlier studies (6). Hepatotoxic agents, the most important being alcohol, are also of importance.

ACKNOWLEDGEMENT

Supported by grants from the Swedish Medical Research Council (Project no. B73-04a-2235-0713).

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INHIBITION OF LEUCOCYTE ALKALINE PHOSPHATASE
BY CYANIDE IN VIVO

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Abstract. The alkaline phosphatase activity of the neutrophilic leucocytes has been determined in rabbits after infusion of large doses of cyanide. A rapid decline in the activity was observed with return to normal values after 3-4 weeks. At the point of maximal suppression the activity could be restored to normal values by infusion of zinc acetate.

Leucocyte alkaline phosphatase (LAP) is a metalloenzyme containing zinc in the molecule as an integrated part of the functional structure, mandatory for the activity of the enzyme (11).

It has been demonstrated that chelation of zinc with various amino acids in vitro completely inhibits the activity of the enzyme (1-5, 6).

The present experiments describe a presumably analogous inhibition of the LAP activity in vivo after i.v. infusion of cyanide ions to rabbits. The effect is rapidly achieved and surprisingly long-lasting with return to normal values after 25-30 days. The in vivo suppressed LAP activity can be restored to normal values by i.v. infusion of zinc. The restoration of the activity to normal values is completed within hours.

MATERIAL AND METHODS

Fourteen 18-20 month New Zealand albino rabbits of both sexes, weighing 1.9-4.1 kg, were used.

Cyanide was given intravenously in an ear vein as solutions of potassium cyanide in sterile 6% glucose. The dose rate was varied to give the appropriate dose starting with a dose rate of 1.5 mg CN/kg b.wt./hour. The infusion was given over 6-hour period with the rabbit lying in a restraint box and using roller type peristaltic pump. The total dose of cyanide given is calculated and given in Table I.

The LAP activity was demonstrated by modified arcody technique (9) developed from the technique originally

described by Kaplow. Blood samples were obtained from an ear vein, fixed and stained. Three hundred cells were differentiated in the scoring process according to the LAP activity and the total and differential scores were calculated.

Zinc was given as an i. single dose of 10 mg of zinc acetate solution containing 2.5 mg Zn⁺⁺/ml.

Leucocyte and differential counts on peripheral blood were performed by standard techniques.

RESULTS

Typical results from individual rabbits are shown in Figs. 1, 2 and 3. On the ordinate is indicated the percentage of the neutrophilic leucocytes with different scores from peripheral blood of the rabbit.

Table I. Total doses of cyanide given and the percentage inhibition of the total score after 6 h

Coefficient of correlation between total dose given and percentage inhibition of total score
-0.60 g, T = 0.228, r = 2.64, 0.01 < p < 0.02

Sex	Weight (kg)	Total dose KCN (10 ⁻³ mol)	Total dose KCN/kg b.wt. (10 ⁻³ mol)	Inhibition of total score after 6 h (%)
♀	2.8	108.4	38.7	34
♀	2.8	139.3	49.8	38
♀	2.5	124.4	49.8	39
♂	2.2	123.0	55.9	46
♀	1.9	119.0	62.6	32
♀	3.1	228.5	73.7	44
♀	2.7	186.6	69.1	38
♀	2.3	118.7	51.6	60
♂	4.1	156.8	38.3	30
♂	3.2	265.4	82.9	90
♂	2.9	211.0	72.8	43
♂	3.2	235.0	73.7	60
♂	2.7	149.3	55.3	40
♂	2.6	143.7	55.3	48

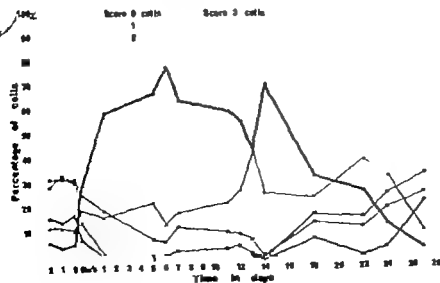
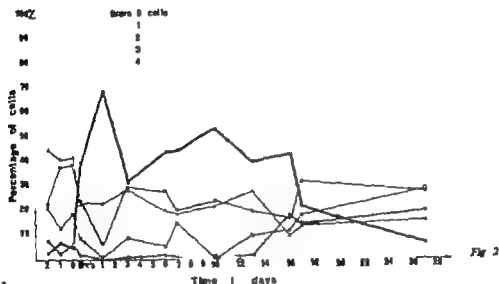
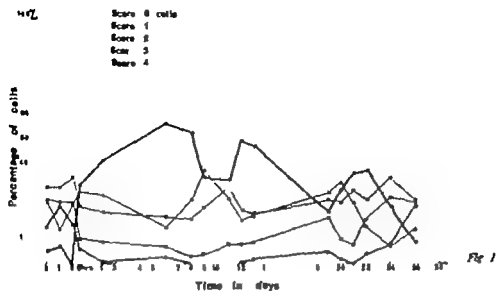


Fig. 3

Figs. 1-3. Variation in LAP activity of the neutrophilic leucocytes of 3 rabbits expressed as the percentage of cells differentiated on basis of "positivity" according to the score system of Hayhoe and Quaghebe (9). Three pretreatment values are obtained and cyanide was given at time zero.

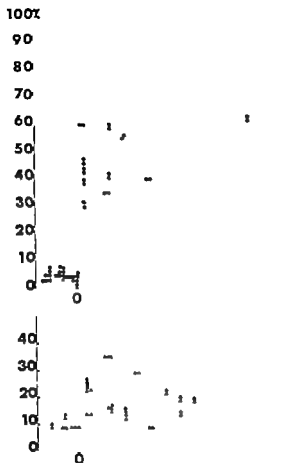


Fig. 4 and b. (Fig. b see next page.) Results from all rabbits, showing, analogous to Figs. 1-3, the variation in the percentage of leucocytes with different degree

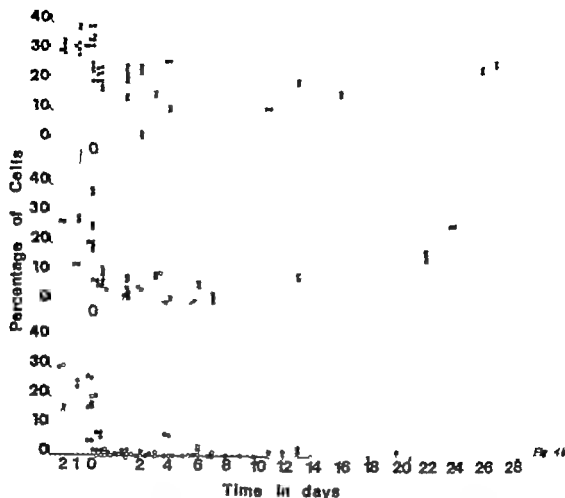
of positivity from zero to plus 4. Symbols correspond to those used in Figs. 1-3.

Fig. 4

bit. Blood was obtained from 2 days before the infusion until return to normal values after 25-30 days. It is apparent that the relative number of cells counted as score zero cells is low, less than 10% in normal rabbit blood, and that the number of these cells increases rapidly to maximum values after a few days with most of this increase completed within 6 hours after the start of the infusion. Similarly the score 4 cells, those demonstrating the highest degree of alkaline phosphatase activity disappear from the circulation within a few days again, with most of the drop within 6 hours. As opposed to the striking changes in these 2 groups of cells there is but a minor drop in the number of score 3 cells after the cyanide infusion and no appreciable change in the score 1 and 2 cells. The percentage inhibition of the total score measured 6 hours after the infusion

was correlated to the total amount of infused CN/kg b.wt. with a correlation coefficient of +0.609 and a p -value between 0.01 and 0.02. All the changes in the LAP activity however have vanished by the end of the observation period. The results from all the experiments are summarized in Fig. 4. Essentially the same pattern of changes is apparent but rather a large spread is noted in the results.

Fig. 5 illustrates the results obtained in 2 out of 4 rabbits given zinc intravenously on day 5 at a time when the suppressive effect of the cyanide dose appears to be maximal. In this Figure the total score is calculated by adding the score of the differentiated cells. It is evident that the infusion of zinc brings about a reversal of the cyanide effect, restoring the LAP activity in the neutrophilic leucocytes to normal within few hours. Total and



differential leucocyte counts were not significantly changed through the observation period.

DISCUSSION

A fairly large number of alkaline phosphatases have been described, showing marked differences between species as well as differences from one organ to another within the same animal (2, 8, 10).

It is, however, apparent that most of these phosphatases contain zinc in the molecule as an integrated part of the structure and necessary for the activity of the enzyme (11, 15, 16).

In addition, magnesium ions seem to be mandatory for the function of a number of these enzymes (14).

Other divalent metal ions, preferentially cobalt, may substitute for zinc and magnesium in vitro,

forming "new" enzymes with specific kinetic characteristics (2, 11, 15).

Chelation by EDTA and a number of amino acids of the divalent metal ions from the incubation medium inhibit the activity in vitro (1, 4, 5, 6, 12).

In animal experiments zinc deficiency has been associated with a low activity of alkaline phosphatase in extracts from various organs (3, 13).

The present series of experiments demonstrates that it is possible to inhibit in vivo most of the leucocyte alkaline phosphatase activity in the neutrophilic leucocytes from rabbits by the infusion of cyanide. The fact that this suppression of the phosphatase activity can be restored by the infusion of zinc makes it likely that the observed effect is mediated via a chelation/complexion of the zinc from the neutrophilic leucocytes with the infused cyanide ions. It was observed that the



Fig. 5 Total LAP score in two rabbits given zinc infusion after maximal suppression of the LAP activity

score 4 cells disappeared rapidly from the peripheral blood and that some of the score 3 cells also vanished, yet without any significant alterations in the total leucocyte count. The percentage of score 1 and 2 cells, however, did not change significantly which may indicate that either some of the leucocytes contain an alkaline phosphatase that is not zinc-dependent but possibly as proposed by Valentine et al. (17), dependent on magnesium, or that both zinc and magnesium-dependent alkaline phosphatases are present in the neutrophil leucocytes from the rabbit. The observation by Diamant et al. (7) of a close correlation between the histochemical determination of the alkaline phosphatases, using the above employed score system, and the biochemical determination of the activity in eluates from the leucocytes ($r=0.8$, $p=0.01$) adds additional credibility to the histochemical methods.

In a number of parallel determinations of the serum alkaline phosphatases it was found that this activity was not affected by the cyanide infusions, as opposed to the activity of the leucocyte phosphatases.

ACKNOWLEDGEMENT

Aided by grants from Carl Schepler and Wilhelmina Foundation.

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THE EFFECT OF ANTICOAGULANTS ON POSTOPERATIVE FIBRINOGEN METABOLISM

Secondary Anaemias XVI

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Abstract. Studies of the effects of dicoumarol and heparin on the metabolism of radioactively labelled fibrinogen given to elderly injured patients subjected to reparative surgery showed that: 1) The actual amount of labelled protein (mg/kg) synthesized and catabolized per day was approximately three times normal, irrespective of whether therapy included or excluded dicoumarol or heparin. The increased rate of synthesis of fibrinogen leads to the observed increased plasma concentrations of fibrinogen. 2) The amount of radioactivity outside the active circulation was increased both in the patients treated with dicoumarol or heparin and in those given no anticoagulants. Similarly the radioactivity in the operated legs as often higher than in the unoperated legs irrespective of whether anticoagulant had been administered or not. The radioactivity outside the active circulation had longer biological half-life than that of the plasma fibrinogen, suggesting that at least some of the non-circulating radioactivity as attached to fibrin even in the patients receiving heparin. The increased rate of synthesis of fibrinogen leads to the observed increased plasma concentrations of fibrinogen.

Studies of the effects of various forms of injury and its surgical treatment on fibrinogen metabolism have indicated that fibrinogen synthesis, its plasma concentration, its rate of catabolism, and probably its rate of conversion into fibrin are increased in injured patients (4). In many elderly patients considerable amounts of radioactivity probably attached to fibrin, are found outside the active circulation (4).

The suggestion from these radioactive studies,

that part of the injected labelled fibrinogen is converted to fibrin, has been confirmed in fatally injured patients by observation at autopsy of deep vein thrombi and microthrombi formed before death in lung tissue (3, 8, 17).

The incidence of death from the effects of deep vein thrombi has been reduced by the administration soon after injury or surgery of one of the anticoagulants, heparin, dicoumarol or phenindione (2, 10, 11, 15, 17).

Since these anticoagulants probably limit the formation of fibrin from fibrinogen, the present purpose was to study the changes in fibrinogen metabolism resulting from the administration of heparin and dicoumarol to injured patients in doses which are clinically effective in reducing the incidence of deep vein thrombi.

MATERIAL AND METHODS

Thirty-five studies were made from two batches of freeze dried fibrinogen (Kabli). Ampoules of reconstituted fibrinogen are labelled with ^{125}I using modification of the iodine monochloride method (6). The labelled fibrinogen solution was either used within 1 hour of preparation or stored at -20°C for not more than 24 hours before use. The specific radioactivities of each preparation were less than $10\ \mu\text{Ci}/\text{mg}$ protein. The average iodine content of all preparations was less than one atom per two protein molecules. The labelled fibrinogen solution ready for injection contained less than 1% of the total radioactivity as uncombined iodine. Between 94 and 97% of the injected radioactive material was clottable. Less than 1.0 ml of labelled fibrinogen solution, containing 5-10 mg fibrinogen, and 15-25 $\mu\text{Ci}\ ^{125}\text{I}$, was diluted with normal saline be-

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Table I. Details of patients studied

Case no.	Age Sex (y)	Operation	Anti-coagulant therapy
<i>Patients with fractures</i>			
1	♂ 68	Fixation of the shaft of femur	None
2	♂ 83	Nailing of the neck of femur	None
3	♂ 88	Amputation of lower right leg	None
4	♀ 83	Nailing of the shaft of femur	None
5	♀ 57	Nailing of the shaft of femur	None
17	♂ 83	Fixation of multiple tibial fractures	None
18	♂ 66	Nailing of the neck of femur	None
19	♀ 70	Nailing of the neck of femur	None
20	♀ 88	Nailing of the neck of femur	None
21	♂ 62	Nailing of the shaft of femur	Dicoumarol
22	♂ 74	Nailing of the shaft of femur	Dicoumarol
23	♂ 76	Nailing of the shaft of femur	Dicoumarol
24	♂ 65	Nailing of the shaft of femur	Dicoumarol
25	♂ 87	Nailing of the shaft of femur	Dicoumarol
26	♂ 49	Nailing of the shaft of femur and plating of tibia	Dicoumarol
27	♀ 44	Nailing of the shaft of femur	Dicoumarol
28	♀ 50	Nailing of the shaft of femur	Dicoumarol
29	♀ 57	Nailing of the neck of femur	Dicoumarol
30	♀ 80	Nailing of the shaft of femur	Heparin ^a
31	♀ 79	Nailing of the shaft of femur	Heparin ^a
32	♂ 83	Nailing of the shaft of femur	Heparin ^a
33	♂ 67	Nailing of the shaft of femur	Heparin ^a
34	♂ 78	Nailing of the shaft of femur	Heparin ^a
35	♂ 82	Nailing of the shaft of femur	Heparin ^a
36	♀ 72	Nailing of the neck of femur	Heparin ^a
<i>Patients requiring arterial bypass grafts</i>			
37	♂ 61	Bypass graft to section of femoral artery	Dicoumarol
38	♀ 51	Bypass graft to section of femoral artery	Dicoumarol
39	♂ 48	Bypass graft to section of femoral artery	Dicoumarol
40	♂ 59	Bypass graft to section of femoral artery	Heparin ^a
41	♀ 36	Bypass graft to section of femoral artery	Heparin ^a
42	♂ 63	Bypass graft to section of femoral artery	Heparin
43	♀ 62	Bypass graft to section of femoral artery	Heparin
44	♂ 59	Bypass graft to section of femoral artery	Heparin
45	♀ 47	Bypass graft to section of femoral artery	Heparin ^a
46	♀ 58	Bypass graft to section of femoral artery	Heparin ^a

20 000 U/day during the 1st and
10 000 U/day during the 2nd week.

fore being slowly injected intravenously into the patient about 24 hours after operative surgery. Oxalated plasma samples were taken from the patient at daily intervals for the first week after injection and then on alternate

days during the second week. The fibrin was heparinized as previously described (7).

When the radioactivity of the isolated fibrin clots had been assayed, its protein concentration was determined by the Biuret method. The fibrinogen concentration in plasma was calculated from this estimate of protein concentration. The radioactivities of the isolated fibrin, plasma and urine were measured in a well type scintillation counter using a single-channel pulse-height analyser and scaler. The random error of counting was always less than 1% and usually less than 1%. After correction of all samples for radioactive decay the counts per ml plasma and the urinary excretion of radioactivity per day were calculated. The rates of loss of radioactivity from plasma and from the whole body and the change in the specific radioactivities of fibrin isolated from plasma, were calculated from regression lines fitted by the method of least squares to the observations plotted on log-linear graph paper.

In fitting these regression lines the results obtained during the first two days after injection were ignored, since mixing of the injected radioactive fibrinogen with the body fibrinogen was not complete until after the time.

The plasma volume was measured with the injected labelled fibrinogen. Plasma volumes subsequent to the first were calculated from the appropriate haematocrit values assuming a constant blood volume as previously described (4, 5). The total plasma fibrinogen radioactivity each day was calculated from the plasma volume and from the counts per ml plasma. The rate of loss of radioactivity from the patients was compared with a scintillation whole body radioactivity counter (16). Daily measurements were usually made with this twin NaI crystal detector. The random error of counting was always less than 0.3% and usually less than 0.1%. A correction was made for the paralysis time of 3 μ sec when the counting rate exceeded 730/sec. As the urine collection was incomplete in some of the patients, the daily change in the quantity of whole body radioactivity was calculated each day from the whole body counter measurements. These daily changes in whole body counts were used in the calculations of catabolic rate by division of the daily loss of radioactivity from the whole body by the whole body content of radioactivity halfway between the times of the whole body counts. The quantity of radioactivity not contained in the circulating plasma was calculated daily by subtracting the total plasma radioactivity from the corresponding whole body content of radioactivity.

The quantity of radioactivity in the injured and re-injured limbs of 11 patients was also measured using the whole body counter. The collimated crystal detectors were placed over each limb 15 cm below the caudal end of the pelvis and at least 30–40 cm from the site of operation.

Clinical material

Studies were made in 35 patients mainly over 50 years of age, with either fractures of the femur or tibia, or with obstructive arterial disease requiring bypass graft. Fuller details of the patients and the operations performed are given in Table I. Some data from patients 1–5 are

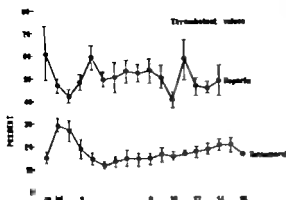


Fig. 1 Mean values and S.E.M. for the thromboplast measurements, expressed as percentages of the expected normal value (following therapy with either dicoumarol or heparin (op = day of operation, inj = day of injection of labelled fibrinogen).

previously been published (4). Fourteen of the patients are given heparin by I. injections at either 4- or 6-hour intervals amounting to about 20 000 U (200 mg)/day starting 3-4 days before operation, on the day of operation and continuing for 7 days. The dose of heparin was reduced to 100 mg/day during the second week after operation. These amounts of heparin were kept small to limit the risk of postoperative haemorrhage. Twelve of the patients were given dicoumarol in doses of 100-200 mg daily for 10-14 days starting 3-4 days before operation. The actual amount of dicoumarol given was varied according to the thromboplast values, the aim being the maintenance of values between 10 and 20% of normal. The actual thromboplast values averaged $14.4 \pm 1.3\%$ of normal in patients given dicoumarol compared with an average value of $65.2 \pm 5.3\%$ of normal in the patients

given no anticoagulants and an average value of $50.0 \pm 5.0\%$ of normal in the patients given heparin (Fig. 1).

All patients received 200 mg NaI orally per day from the day before injection of radioactivity until the end of the study to block thyroid uptake of radioactive iodine. All operations for fixation of the fractured femur are done by the same two surgeons, approximately one week after injury and bleeding during the operations was estimated to be 200-400 ml. The arterial bypass operations were also done by the same surgeons, under tourniquet, with insignificant blood loss. All wounds healed by first intention, and there are no clinical signs of postoperative haematomas except in case 40, in whom 250 ml blood were removed from the operation site one week after operation.

Clinical signs of pulmonary embolism were seen in cases 4 and 19 two weeks after the operation. There were no clinical signs of wound infection or thromboses in the other patients. Mobilization exercises were started on the day after operation both in the patients with femoral fractures and in those requiring arterial bypass grafts.

RESULTS

Inspection of the results of various aspects of the metabolic study revealed insignificant differences between the results from patients with fractures and those requiring arterial bypass grafts who received the same anticoagulant. The results in the two groups of patients given dicoumarol have, therefore, been combined. A similar comparison of results was made in the two groups of patients given heparin.

Elimination of labelled fibrinogen from the circulation and the whole body

Single exponential rates of decrease of the total plasma content of radioactivity of the whole body

Table II. *Biological half-lives (means \pm S.E.M.) of labelled protein (fibrinogen) in plasma and the whole body and the specific radioactivities of isolated fibrin*

	Time after operation (weeks)	N	Half-life (d.)		
			Whole body	Plasma	Specific radioactivity
Normal persons aged 23-53, expected values		35	(4.14) ^a	4.14 ± 0.56	(4.14) ^a
Elderly injured patients	1	9	4.1 ± 0.16	3.0 ± 0.13	3.2 ± 1.09
Not receiving anti-coagulants	2	4	4.5 ± 0.31	3.9 ± 0.34	
Receiving dicoumarol	1	12	4.0 ± 0.21	2.8 ± 0.16	3.1 ± 0.12
	2	4	4.2 ± 0.36	3.5 ± 0.22	
Receiving heparin	1	14	4.2 ± 0.15	3.0 ± 0.17	3.3 ± 0.24
	2	5	4.5 ± 0.18	4.2 ± 0.32	

^aPresumed values derived from plasma half-life (19).

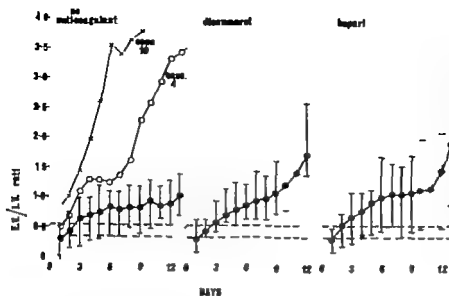


Fig. 2. Distribution of radioactivity between the non-circulating or extravascular (E.V.) and the intravascular (I.V.) compartments in the three groups of patients. Data labelled cases 19 and 4 are from individual patients. The other lines show average values and the range of observations. The expected normal range of values for the E.V./I.V. ratio lies between 0.35 and 0.55.

content of radioactivity and the specific radioactivity values of fibrin isolated from serial plasma samples were observed in most patients. When biphasic exponential rates were observed the earlier rate was used in the calculation. The average biological half-lives calculated from these exponential rates of decrease and the S.E.M. values, in patients receiving either heparin or dicoumarol or no anticoagulants, for the measurements of whole body radioactivity plasma radioactivity and specific radioactivity values are shown in Table II.

The average biological half-lives of the whole body radioactivity are the same in the injured elderly people as those previously published for young controls (19). Simultaneously however the average biological half-lives of the plasma radioactivity and the specific radioactivity values are shorter in each group of elderly patients than in normal persons. The average rate of disappearance of radioactivity from the plasma was always greater than that from the whole body irrespective of the form of therapy. These differences, between the injured elderly and the controls as well as between the whole body and the plasma, in the rates of disappearance from the plasma are highly significant in each group of patients ($p < 0.001$). In contrast, there was no significant difference between the groups given heparin dicoumarol or no anticoagulants in the average biological half-lives of radioactivity in the whole body or in the plasma, or in the specific radioactivities.

The amount of radioactivity not contained in circulating plasma

Two patients (nos. 4 and 19) not receiving anticoagulants showed amounts of radioactivity outside the circulating plasma which exceeded the intravascular content by more than three times. These patients had clinical evidence of thromb. All other patients, including those receiving dicoumarol and heparin, also showed smaller although abnormally large amounts of radioactivity outside the circulating plasma (Fig. 2). Although the mean thrombotest value in the untreated group was 65.2 ± 5.3 and in the dicoumarol-treated 14.4 ± 1.3 significant correlations were found, neither in each group nor in the two pooled groups, between the thrombotest and any of the fibrinogen parameters.

The catabolic rate of fibrinogen

As estimates of the catabolic rate based on changes in the level of plasma radioactivity alone have been shown to be invalid (see Discussion), estimates were based on changes in the whole body content of radioactivity. In all three groups of elderly patients the catabolism (mg/fibrinogen/kg b.wt. catabolized each day) was increased compared with that previously observed in controls (Table III). This increase of catabolism could not be prevented by giving anticoagulants.

On an average the rate of catabolism in the patients given dicoumarol or no anticoagulants was greater during the first week after operation than during the second week (Table III).

Table III *Catabolic rates of the whole body content of labelled protein (fibrinogen) (means \pm S.E.M.)*

	Time after operation (weeks)	N	Catabolic rate	
			% of body content/d.	g/kg b wt/d.
Normal persons aged 23-53 (19), expected values		33	17.3 ± 0.07	0.028 ± 0.009
Elderly injured patients	1	9	18.0 ± 0.75	0.10 ± 0.01
Not receiving anticoagulants	2	4	16.1 ± 0.51	0.11 ± 0.02
Receiving dicoumarol	1	12	17.6 ± 0.63	0.10 ± 0.01
	2	4	15.3 ± 1.16	0.10 ± 0.02
Receiving heparin	1	14	16.8 ± 0.63	0.10 ± 0.009
	2	5	17.4 ± 1.16	0.11 ± 0.02

Measurements of plasma fibrinogen concentration

In all three groups of patients the average plasma fibrinogen concentration lay between 0.48 and 0.59 g % which is greater than the values found in young normal persons (Table IV).

Calculation of the limits to the rate of synthesis of fibrinogen

Entry of newly synthesized unlabelled fibrinogen into the plasma lowers the specific radioactivity of the circulating fibrinogen. Loss of fibrinogen from the plasma, on the other hand, does not affect the specific radioactivity of the remainder. With due allowance for changes in size of the circulating fibrinogen pool, these two principles allow limited deductions about the daily rate of synthesis of fibrinogen. Considering only the circulating fibrinogen, a maximum estimate of daily synthesis was derived from the theoretical increase

of the pool size which would produce the observed fall in specific radioactivity. A minimum estimate of daily synthesis was provided by the difference between the final pool size and the amount of fibrinogen which must have remained from the previous day to provide the measured specific radioactivity. Average values for the maximum and minimum synthetic rates have been calculated for the three groups of patients given labelled fibrinogen (Table IV).

In each group of elderly patients the rate of synthesis of fibrinogen during the first week after operation was increased to approximately twice the rate of synthesis observed in the normal young persons. During the second week this rate of synthesis decreased to nearer the normal range. The rate of synthesis in normal young persons has been used in Table IV because normal values for the rate of synthesis of fibrinogen in elderly persons do not appear to be available.

Table IV *Fibrinogen synthesis and plasma fibrinogen concentrations (means \pm S.E.M.)*

	Time after operation (weeks)	N	Fibrinogen concentrations (g %)	Rate of synthesis (g/kg/d)	
				Max.	Min.
Normal persons aged 23-53, expected values		35	0.28 ± 0.07	0.035^a	0.015^a
Elderly injured patients	1	9	0.53 ± 0.04	0.076 ± 0.01	0.039 ± 0.009
Not receiving anticoagulants	2	4	0.59 ± 0.05	0.045 ± 0.009	0.033 ± 0.009
Receiving dicoumarol	1	12	0.53 ± 0.02	0.068 ± 0.006	0.032 ± 0.004
	2	4	0.48 ± 0.05	0.055 ± 0.006	0.045 ± 0.007
Receiving heparin	1	14	0.51 ± 0.03	0.071 ± 0.008	0.055 ± 0.006
	2	5	0.56 ± 0.04	0.058 ± 0.014	0.048 ± 0.011

Values taken from Davies et al. (7)

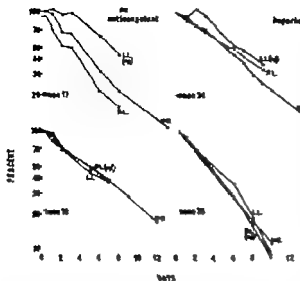


Fig. 3 Rate of change (as percentage of the initial observation) of the whole body content of radioactivity (W.B.) and measurements of limb radioactivity in 4 patients, two of whom showed increased amounts of radioactivity in the limbs that had been surgically repaired (cases 17 and 34). L.L. = change in the radioactivity content of the left leg, R.L. = change in the radioactivity content of the right leg, op. = surgical repair of the limb.

Levels of radioactivity in the unoperated and operated limbs

The whole body counter measurements of the amounts of radioactivity in the operated limbs and uninjured contralateral limbs have been expressed as percentage of the initial observation made within one hour of injection of the labelled fibrinogen. These percentage changes in the leg activities corrected for radioactive decay have been compared with the total body counts (Fig. 3). Each of the four patients on whom measurements were made in the untreated group (1) had a slightly longer half-life in the operated leg than in the unoperated, but none of these patients had the 15% difference in activity between the legs suggesting a thrombosis. The patient with the tibial fracture however had an operation haematoma with much radioactivity.

Of the patients treated with heparin the leg radioactivities were measured in 7 one of whom (case 34) had definite signs of thrombosis and two had borderline values, all in the operated leg. In this group there was no systematic difference in half-life between the operated and the unoperated legs.

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DISCUSSION

Previous studies have shown that the labelled fibrinogen used in this study has a biological half-life which is not significantly different from that of unlabelled fibrinogen (4.5-7).

In the three groups of elderly patients in this study the average biological half-life of the plasma radioactivity was usually less than the mean value of 4.14 ± 0.56 days observed in 35 healthy young and middle-aged adults (19). There was little difference in either the mean values or the range of the observations of the biological half-life of plasma radioactivity between the patients treated with either heparin or dicoumarol and those given no anticoagulants. The fact that even some patients given anticoagulants had signs of fibrin deposition in the operated leg also suggests that anticoagulants have only a small effect on the postoperative fibrinogen metabolism. This failure of dicoumarol to alter the rate of disappearance of radioactivity from the plasma confirms the findings reported by Adelson et al. (1) from 3 patients given dicoumarol for 4-6 weeks after myocardial infarction. Nor did heparin given to 5 normal subjects by Tytgat et al. (19) and to many dogs by Lewis et al. (12) change the biological half-life of fibrinogen from that observed in normal men and dogs.

The slower rate of disappearance of radioactivity from the whole body than from the plasma, observed in the patients treated with or without anticoagulants, may be due either to the transfer of radioactivity from the circulating plasma to a site which is outside the normal circulation or to the conversion of soluble fibrinogen into insoluble fibrin within the vascular system. Neither of these possible changes appeared to be affected by the administration of dicoumarol or heparin. These results confirm the findings described by Davies et al. (4) that the non-circulating fibrin or fibrinogen survives longer than the circulating fibrinogen. The longest survival of whole body radioactivity was found in the two patients who had the largest amount of radioactivity outside the circulating plasma. Both patients had signs of lung emboli 2 weeks after the operation.

Much less radioactivity was found outside the active circulation in the other patients (Fig. 2). Even in these patients, however, the amount of radioactivity outside the active circulation was greater than that found in normal young persons

and in young patients with knee injuries (4). As some of this non-circulating radioactivity could have been due to blood clots at the site of the surgical repair an attempt was made to reduce the significance of this possibility by delaying the injection of labelled fibrinogen until the day after operation.

The estimates of the catabolic rate of fibrinogen have been based upon the loss of radioactivity from the whole body rather than only from the plasma, since the whole body counts reflect changes in both the circulating and non-circulating radioactivity. The catabolic rate of the whole body content of labelled protein (percent age/day) was not significantly affected by either of the administered anticoagulants. The catabolism in mg/kg/day however was of course considerably increased in all three groups of patients because of the elevated body content of fibrinogen.

The increased plasma concentrations of fibrinogen (averaging $1\frac{1}{2}$ to 2 times the expected normal values in young persons) were virtually the same in all groups of patients and are probably a response to injury. They are probably a result of the increased rates of synthesis observed in each group of elderly patients. Marked increases in fibrinogen concentration have been described in other patients with moderate and severe injuries (9) and in patients with burns (7). It is possible that the increased reticuloendothelial activity described after injury may explain both the increase in fibrinogen synthesis, which has been suggested to take place in the reticuloendothelial cells, and the anaemia of injury (13-20).

The increased rates of synthesis also explain the reduced biological half-lives of the labelled protein expressed as specific radioactivities (counts/mg fibrin), since the newly synthesized unlabelled fibrinogen diluted the existing labelled plasma fibrinogen levels at a greater than normal rate. No data appear to be available showing whether these increased rates of synthesis of fibrinogen in elderly patients are significantly different from those observed in normal elderly individuals.

ACKNOWLEDGEMENT

Supported by the Swedish Medical Research Council (grant no K70 19X 2340-03A).

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SURVIVAL OF DFP-LABELLED ERYTHROCYTES IN URAEMIC PATIENTS DURING EXTRACORPOREAL IRRADIATION OF THE BLOOD

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Abstract. The survival time of erythrocytes labelled with ^{51}Cr -diisopropylthiophosphatate (DFTP) was investigated in uraemic patients on chronic dialysis treatment. Twelve patients were studied, eight survival studies were performed in patients not undergoing extracorporeal irradiation of the blood (ECIB), and 7 survival studies were made in connection with ECIB treatment. The mean erythrocyte survival time was calculated by linear regression analysis of the results obtained 5-36 days after labelling and extrapolation of the curve to the time of zero activity. In all the patients the erythrocyte survival was shortened; in patients not undergoing ECIB the median survival time was 55 days (range 44-62). The survival curves indicated an accelerated and predominantly age-dependent erythrocyte destruction. ECIB was generally given from day 18 to day 36 after labelling of the erythrocytes. The accumulated radiation dose given to the blood ranged from 46 900 to 70 000 rads. There

was no demonstrable influence of ECIB on red cell survival as estimated from the slopes of the individual survival curves before and during ECIB. However, the reduced life span of the erythrocytes in these uraemic patients made it difficult to evaluate superimposed haemolytic component. The fact that the median Hb concentration in the blood decreased slightly but significantly in further 15 patients during ECIB indicates that ECIB may induce slight reduction in red cell survival in severely uraemic patients. This has, however, few clinical consequences.

Extracorporeal irradiation of the blood (ECIB) has been used as an adjunct to conventional immunosuppressive therapy in patients undergoing renal transplantation (17). The aim of ECIB is to achieve pronounced lymphocytopenia of considerable duration. In previous study (16) it was shown that the duration of lymphocytopenia following ECIB depends on the accumulated irradiation dose given to the blood, usually expressed as the mean cumulative erythrocyte dose (MCED). After a MCED of 50 000 rads the lymphocyte

concentration remained at 30% of the pretreatment level for 8 months, whereas the lymphocyte concentration increased 3 months after cessation of ECIB with a MCED of 20 000 rads.

Erythrocytes are much more resistant to irradiation than lymphocytes, but with the large doses employed in ECIB an increased destruction of red cells may be anticipated. Sipe et al. (15) observed pronounced haemolysis in animals following high doses of ECIB. Studies with ^{51}Cr labelling have shown a decreased survival in vivo of normal human erythrocytes after in vitro irradiation with 35 000 rads (14). In patients with chronic lymphocytic leukaemia undergoing ECIB an increased loss of ^{51}Cr activity from the blood was observed when MCED reached 18 500-34 000 rads (1). A decreased Hb concentration and reticulocytosis were observed in 4 of 5 uraemic patients during ECIB with MCED of 20 000-35 000 rads (13). Erythrocyte survival studies have, however not previously been carried out in uraemic patients treated with ECIB as a preparation for kidney transplantation.

MATERIAL AND METHODS

Patients

Erythrocyte survival studies were performed in 12 uraemic patients. Pertinent clinical data are shown in Table I. Ten of the patients were treated by haemodialysis twice weekly (nos. 1-10), two by peritoneal dialysis once weekly (nos. 11 and 12). The artificial kidney used was the Kal system for three of the patients (nos. 6, 8 and 10). The amount of blood lost in the dialyser was about 200 ml/month. The remaining seven patients were dialysed in the Gambro system; the amount of blood lost in this system was about 50 ml/month. About 200-

Table I. Clinical data on 12 uraemic patients studied by ^{51}Cr labelling

Pat. no.	Age (y)	Sex	Primary kidney disease	Duration of uraemia (mo.)	Duration of dialysis (mo.)	Bilateral nephrectomy	Status during last two months before ^{51}Cr labelling		
							BP (mmHg)	Mean blood urea (mg/100 ml)	Blood transfusions (ml/2 mo.) ^a
1	41	♀	Polycystic disease	34	3	—	110/70	90-90	300
2	35	♂	Chronic pyelonephritis	15	5	—	120/80	60-90	1785
3	41	♀	Polycystic disease	18	8	—	120/80	90-90	695
4	37	♀	Chronic glomerulonephritis	5	3	—	130/110	60-100	1540
5	44	♂	Chronic glomerulonephritis	52	35	+	110/70	90-90	1500
6	29	♀	Chronic pyelonephritis	17	1	—	130/90	100-125	640
7a	43	♀	Chronic pyelonephritis	13	13	+	110/70	90-100	1440
7b				24	24	+	110/70	90-100	2110
8	36	♀	Nephrosclerosis	13	12	+	110/70	75-90	1200
8b				5	4	+	160/80	75-90	1170
8c	37			34	3	+	110/80	75-90	1120
9	27	♂	Chronic glomerulonephritis	3	3	—	140/100	90-70	990
10	35	♀	Chronic glomerulonephritis	7	5	—	180/110	90-60	1385
11	48	♀	Chronic glomerulonephritis	5	1	—	140/100	75-130	740
12	20	♂	Chronic pyelonephritis	19	2	—	140/90	100-130	300

^a Creatinine clearance < 20 ml/min.

^b Leucocyte-poor blood, mean haematocrit 30.

300 ml of blood were drawn per month for laboratory analyses. This amount was kept constant during the different phases of the study. None of the patients had severe bleeding episodes, but in two women (nos. 7 and 8) continuous vaginal bleeding increased the requirement for red transfusions.

Six labelling studies were performed in patients not undergoing ECTB (nos. 1-6). Two patients were studied during as well as without ECTB (nos. 7 and 8): the latter was studied during two different ECTB periods. Five survival studies were made in patients during ECTB treatment (nos. 9-12).

ECTB

The technique of ECTB has previously been described in detail (16). A ^{60}Co or ^{137}Cs source was employed. For patients 7, 8, 10, 11 and 12 the median duration of ECTB was 90 hours (range 75-111) given over a period of 19 days (range 11-27). The median transit dose was 360 rads (range 300-400) and the MCED 63 400 rads (range 46 500-70 700). Patient 9 was treated according to different ECTB schedule—163 hours over period of 42 days with a transit dose of 100 rads and a MCED of 17 700 rads. MCED was calculated as described by Weeke (16): this calculation applies only in cells that are in the blood throughout the entire period of irradiation.

Labelling, blood sampling and counting techniques

Erythrocytes were labelled by intramuscular injection (on day 0) of 100 μCi ^{51}Cr DFP specific activity 200-300 $\mu\text{Ci}/\text{mg}$ DFP (The Radiochemical Centre Ltd., Amersham). Samples of 2 ml heparinized blood were drawn at frequent intervals. Blood samples were treated in the following way: Haematocrit was determined (Ecco haematocrit centrifuge). Erythrocytes were washed three times and resuspended in isotonic saline and the haematocrit of the suspension was measured. The suspension may be kept for at least 14 days without any change before preparation for liquid scintillation counting. This preparation was performed in TrCarb counting lab as follows.

1) 200 μl erythrocyte suspension (i.e. 70-90 μl erythrocytes) were pipetted into the bottom of the vial. 2) 1 ml of a 50/50 solution (v/v) of SoleneTM 100 (Packard) and Dioxane (G.R.), 3) 0.5 ml hydrogen peroxide 35%, were pipetted into the vial. After 1) and 3) each vial was immediately put into a waterbath of 40°C for 10 min. 4) Then 15 ml scintillation liquid (InstaGel, Packard) are added to the vials, which were closed and thoroughly shaken. Samples are kept at 4°C in the dark until counting.

Counting as done in a TrCarb liquid scintillation spectrometer model 3320. ^{51}Cr is β -emitter (17 MeV).

Table II Erythrocyte survival data in 12 uraemic patients in relation to ECIB

Pat. no.	Duration of		Mean cumulative erythrocyte dose (rads)	Haematocrit		Blood transfusions during study ^a (ml)	Mean erythrocyte survival (d.)	II/I
	DF ⁵² P study (d)	ECIB (d)		Beginning of study	End of study			
1	45	—	0	25	24	290	60	1.2
2	36	—	0	22	22	900	62	0.8
3	49	—	0	24	24	870	60	1.2
4	45	—	0	24	24	150	50	0.8
5	49	—	0	22	23	1 245	56	1.0
6	35	—	0	23	23	370	54	1.2
7	45	—	0	18	18	1 840	48	1.3
7b	33	19-40	46 500	18	20	1 570	38	0.5
8	49	—	0	18	23	1 700	44	1.2
8b	35	19-36	53 500	20	20	1 045	41	0.8
8	34	18-29	70 000	21	16	1 410	39	2.1
9	42	13-55	17 700	20	20	1 350	64	
10	49	19-36	59 700	23	20	0	44	1.0
11	45	14-34	68 200	24	22	1 420	37	1.0
12	43	18-39	67 100	24	20	1 665	31	1.3

^a Leucocyte-poor blood, mean haematocrit 80.

^b For explanation see Material and methods and Fig. 2.

and has half-life of 14.3 days. The spectrometer had been calibrated in the following way. The amplification giving optimal counting rate of standard samples of DF⁵²P prepared as described above was determined. (This optimum amplification is little higher than for completely unquenched samples.) At this amplification the channel was set from "zero" in order to minimize the effect of quenching on counting efficiency. Quenching has been controlled by remeasurement with an external standard; quench correction was not necessary. The variation coefficient of the whole procedure was 4% (not counts per sample were not below 3 000).

Calculations

The results were expressed as counts per ml/ml blood and plotted in a linear scale versus time. As the main part of the curve occurred linear, the mean survival time of the erythrocytes (I) was estimated in the following way. A linear regression analysis was made, including all results in the interval 5-36 days after labelling. The extrapolated cross-section of this line at the time axis is referred to as T.

I: an attempt to assess the existence of any change in the slope of the survival curve in relation to ECIB, two sections of the curve in the individual patient were compared: pre-ECIB section (I) from day 5 to the starting of ECIB, and an ECIB section (II) from the day when cumulative radiation dose of 20 000 rads was reached and to the end of ECIB (Fig. 2). For each section the slope was determined by linear regression using the method of least squares, and changes in the slope were expressed as the ratio between the slopes (II/I).

RESULTS

The results of the erythrocyte survival studies are presented in Table II. In Fig. 1 the survival of

DF⁵²P labelled erythrocytes in a patient who did not receive ECIB treatment is shown. In four patients an initial steep decline in activity was seen. In three of these cases the haematocrit fell during the same period, in one (no. 12) the haematocrit went up due to transfusions. In all other patients the initial part of the curve showed either no decrease or a decrease in activity not different from the rest of the curve. This is in accordance with the results of other investigators (2, 11). Throughout the main part of the study the curve seemed linear. In some patients (nos. 1, 3, 5, 7a, 8a, 11) a tailing of the final part of the survival curve from day 36 gives the graph a curvilinear appearance (Fig. 1).

The survival of erythrocytes was shortened in all patients. In patients not treated with ECIB the median of mean survival times was 55 days (range 44-62). As regards the blood loss due to dialysis and to sampling for laboratory studies, a calculation shows that the maximal effect of these procedures would be a shortening of mean erythrocyte survival by 8 days.

Fig. 2 shows the survival of erythrocytes in a patient in relation to ECIB treatment. Excluding patient 9 who received a MICE below 20 000 rads, the median survival time of erythrocytes in ECIB-treated patients was 40 days (range 37-51). This is significantly lower than T for the non-ECIB-treated patients ($p < 0.01$) but the differ

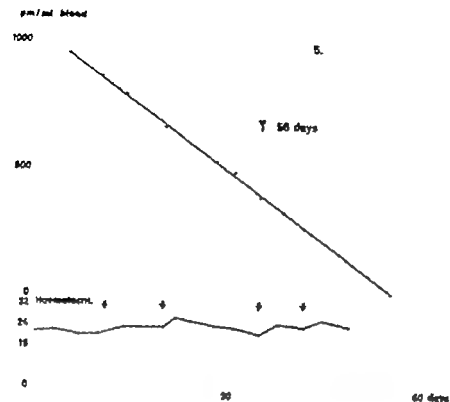


Fig. 1 Survival of DF^{57P} labelled erythrocytes in patient 5. There seems to be a falling of the curve after day 56. An arrow indicates transfusion of 300 ml leucocyte-poor blood, serum haematocrit 80 %

ence between the two groups appeared already in the pre-ECIB section (I) of the survival curves ($p < 0.05$). The calculation of ratios between slope

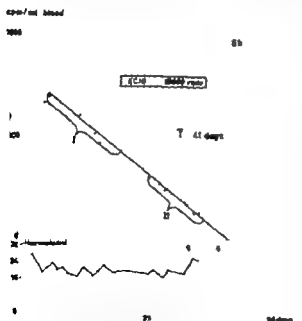


Fig. 2. Survival of DF^{57P}-labelled erythrocytes in patient 8 in relation to ECIB treatment. There is no difference in the slopes of part I (the pre-ECIB section) and part II (the ECIB section) of the curve. Symbols as in Fig. 1.

II and slope I gave no indication of influence from ECIB upon erythrocyte survival. The ratio ranged from 0.53 to 1.26 in the non-ECIB-treated patients and from 0.47 to 2.06 in the ECIB-treated. Calculated by means of the rank sum test for independent samples this difference was not significant ($p > 0.1$).

DISCUSSION

Erythrokinetic studies in severe uraemia present difficulties due to the non-steady state. The reduced erythropoiesis (4) necessitates transfusions. Blood losses during dialysis occur although the amount lost is very small with modern techniques, and besides spontaneous haemorrhage is encountered.

For the study of erythrocyte life span, labelling with DF^{57P} is preferable. Its main advantage over the ⁵¹Cr technique is that after the first few days no elution of label occurs. As a consequence the problem of changes in the rate of elution of label due to the ECIB which cannot be excluded with ⁵¹Cr does not occur with DF^{57P}. The routine application of this method is facilitated by the liquid scintillation counting employed in the present study.

The results indicate that all patients had a reduced red cell survival time, on the average about 50% of normal. This is in accordance with the results of Eachbach *et al.* (5) who also used $DF^{32}P$ -labelling in uraemic patients in chronic dialysis. Some earlier studies, in which labelling with $Na_2^{51}CrO_4$ was employed, have shown normal erythrocyte survival in many uraemic patients (9, 12), and it is generally accepted that there is a poor correlation between the degree of haemolysis and the severity of uraemia (4, 8).

The initial decline in blood activity in four patients might be explained by initial elution of tracer from intact red cells (7). Another possibility would be the presence of two or more erythrocyte populations, transfused and autochthonous cells, one with a shorter life span, simultaneous labelling with two isotopes might elucidate this question (11). However in these four patients the concomitant variations in haematocrit preclude a closer analysis.

The main part of the survival curve in this study seemed linear in a non-transformed plot. Under certain conditions, primarily a constant red cell volume, this pattern of destruction is most often interpreted as cell death from senescence. The linearity of the curve is surprising. Both autochthonous and transfused cells have been labelled and these two erythrocyte populations do not necessarily behave in the same way. Furthermore this interpretation of the curves is in contrast with the generally accepted concept of random destruction of erythrocytes in uraemia (6). This concept has been based upon studies with cross-transfusion using the Ashby technique (10) or by comparing the results obtained in simultaneous studies with the Ashby technique and Cr labelling (3). However it is difficult to draw firm conclusions from these results, because a component of immune haemolysis is impossible to exclude in the Ashby technique, in particular in previously multitransfused patients. Due to continuous elution of tracer from intact cells the

Cr method does not give reliable information about the pattern of cell destruction. The $DF^{32}P$ method should be more applicable for this purpose.

A reduction of erythrocyte survival might have been expected during treatment with accumulated radiation doses as employed in this study. Several factors, however, would tend to minimize such

an effect. Due to their uraemic state, these patients have a reduced erythrocyte life span, and it is difficult to evaluate a superimposed haemolytic component. In addition, the reduced life span of the red cells entails a preponderance of young cells which are less radiosensitive (14). The increased turnover rate of erythrocytes means that a smaller proportion survives in the circulation during the entire irradiation period.

In the present study there was no demonstrable influence of ECIB on red cell survival. In order to further elucidate this question, the data on 15 consecutive uraemic patients were analysed before, during and after ECIB. These patients did not receive any transfusions median MCHC was 52 020 rads (range 49 500–63 200) and median duration of ECIB 16 days (range 8–20). The median Hb concentration before ECIB was 4.5 mmol/l (range 3.3–5.7) during ECIB 4.2 (3.1–5.4) and after ECIB 3.8 (2.9–5.2). Employing the Wilcoxon matched-pairs signed-ranks test, this decrease in Hb concentration during and after ECIB was significant ($p < 0.01$). Thus it may be concluded that a slight reduction in erythrocyte survival during ECIB may possibly occur however this is of minor clinical importance.

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COAGULATION STUDIES IN PATIENTS TREATED WITH DEFIBRASE

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Abstract. Coagulation studies have been carried out on 33 patients who were anticoagulated by induced hypofibrinogenaemia, by using Defibrase, purified thrombin-like enzyme from the venous of *Bothrops atrox*. Patients are given 50-150 µl Defibrase/kg b.wt./day maintaining fibrinogen concentrations around 0.7 mg/ml plasma for 4-21 days. Soluble fibrin appeared in the circulation during the initial phase of treatment. Though not demonstrable in plasma an increased fibrinolytic activity as indicated by an increased amount of fibrin(ogen) degradation products in the serum and partial depletion of the plasma concentration of plasminogen and α_2 -macroglobulin. The degree of plasminogen depletion was correlated to the degree of defibrination. During Defibrase treatment the concentration of factor XIII fell, however no reduction was observed for the concentrations of coagulation factors II, V, VIII, X and XII or for the prothrombin-proconvertin test and Normovist values. The concentration of antithrombin III was unaffected by the Defibrase treatment. A sensitivity reaction was suspected in one patient, but in some of the patients was an increased capacity to neutralize Defibrase demonstrated. The results are discussed with regard to haemostasis and anticoagulant effect. It is concluded that Defibrase treatment is comparable to other common anticoagulant treatments.

As a method for the management of thrombotic disorders therapeutic defibrination was introduced in 1968 by Bell et al. (6) and Sharp et al. (44). Defibrination was accomplished by i.v. infusion of Arvin, the purified thrombin-like enzyme from the venom of *Agkistrodon rhodostoma* (21). Defibrase another thrombin-like enzyme separated from the venom of *Bothrops atrox* (28) has similarly been used for therapeutic defibrination (17, 18).

Arvin and Defibrase clot fibrinogen by splitting

Defibrase preparation contains only the thrombin-like enzyme from *Bothrops atrox* venom and is meant for therapeutic use. Synonymes Defibrol and earlier also Repulse-DEF Repulse R is an equivalent preparation but meant for laboratory use only.

off fibrinopeptide A (11, 25) and the enzymes are not inhibited by heparin (5, 14). Other peptides, however may also be split off from the fibrinogen molecule by these enzymes (24, 32). In vitro it was further observed that Defibrase (29) but not Arvin (4), activates the fibrin stabilizing factor (FSF or factor XIII). In spite of this both Arvin and Defibrase clots have in vitro been demonstrated to have an increased lability to fibrinolysis compared with clots formed by thrombin (30). Other coagulation factors, however seem to be unaffected by these enzymes (5, 6, 17, 18, 44).

In this paper are reported the results of coagulation factor analysis, determinations of fibrin(ogen) degradation products (FDP) fibrinolytic activity plasminogen, α_2 -macroglobulin and antithrombin III during treatment with Defibrase. The occurrence of fibrinemia during defibrination was tested by the ethanol gelation test and by N terminal amino acid analysis. Inhibition of Defibrase by serum from normal subjects and Defibrase-treated patients was also tested. The results of studies made on 33 patients undergoing therapeutic defibrination with Defibrase for various thrombotic disorders are reported.

MATERIALS AND METHODS

Defibrase supplied by Dr K. Stocker, Pentapharm, Basle, Switzerland. Batches 403, 416, 460, 477 and 500: sterile preparations with the active enzyme dissolved in 0.9% NaCl containing 0.3% phenol (batches 403, 416 and 460) or 0.3% chlorobutanol (batches 477 and 500). The thrombin-like activity of the preparations was assayed as an albumin-containing test system with bovine fibrinogen as substrate (12) and found to correspond to 3-4 NIH units/ml (9).

Thrombin (bovine), essentially free from plasminogen, prepared according to Blombäck and Yamashita (16).

Plasminogen (human) supplied by Dr P. Wåhlén, Dept.

of Medical Chemistry Umeå University Umeå, Sweden, highly purified according to Wallén and Wiman (48).

Fibrinogen (human), free from plasminogen, prepared according to Bergström and Wallén (8) and used for preparation of fibrin plates for test of spontaneous fibrinolytic activity in euglobulin precipitates.

Agar from Difco Laboratories, Detroit, USA.

Horseradish peroxidase (Bodrops serum (polyvalent), rabbit antiserum against human FDP II and E from Berlingwerke, Marburg/Lahn, West Germany.

Rabbit immunoglobulin against human fibrinogen, and rabbit immunoglobulin against human α_2 -macroglobulin from Brostex, Copenhagen, Denmark.

Rabbit antiserum against human antithrombin III from Nyrsgaard, Oslo, Norway.

Rabbit antiserum against human plasminogen. Plasminogen was dissolved in 0.15 M NaCl containing 30 mg Benzyl penicillin/ml to a concentration of 10 mg/ml. Equal volumes of this solution and Freund's incomplete adjuvant (Difco Laboratories, Detroit, USA) were thoroughly mixed and injected into the footpads of rabbits (0.15 ml/foot pad). Ten weeks later second dose was given in the same way and the rabbits were bled 3 weeks thereafter.

Clinical material and therapeutic procedure

The data were obtained from 33 patients treated with Defibrase for 4–14 days. Seven data on 13 of these 33 patients have been reported earlier (17, 18). Eleven patients were treated for acute leg thrombosis and one for fresh thrombosis of the axillary vein; positive finding on functional ascending phlebography was the criterion for the diagnosis. One patient was afflicted with polycythemia vera complicated by recurrent thrombotic episodes. Five patients were treated for acute central retinal vein thrombosis. Fifteen patients were anticoagulated with Defibrase at vascular surgery including thrombectomies on the aorta side, thromboendarterectomies and by-pass operations using autologous venous grafts.

Defibrase was in all cases administered intravenously in 100 ml physiological saline during one hour. Prior to the first infusion of Defibrase, an i.v. injection of heparin (10 000–15 000 IU) was given to the patients afflicted with acute thrombosis in order to achieve an immediate anticoagulant effect.

The initial dose of Defibrase was 10–40 μ l/kg b.wt. in the first 12 patients treated. While the remaining patients were given an initial dose of 50 μ l/kg b.wt. Fibrinogen values were determined approximately 18 hours after each dose of Defibrase and subsequent doses of Defibrase were adjusted according to the values obtained. When the required dose of Defibrase exceeded 70 μ l/kg b.wt./day it was administered in 4 infusions per day. Further adjustment of Defibrase doses was then done according to fibrinogen values obtained 6–12 hours after previous infusion. The maximal dose given per day was 154 μ l/kg b.wt. Dicumarol treatment was instituted 4–4 days before cessation of Defibrase treatment.

Most patients treated in connection with surgery were defibrinated 3–14 days before the operation. This was done in order to avoid surgery during the initial phase of Defibrase treatment when the concentration of FDP as at its maximum and could interfere with hemostasis. Transfusion of blood cell concentrates and albumin are

given to these patients in case of anemia and/or hypotolemia.

Laboratory methods

Blood sampling. Blood samples were taken before Defibrase treatment at various intervals during treatment and for 1–8 days after cessation of treatment. Samples were preferably taken by vein puncture but mostly had to be drawn through permanent polyethylene vein catheters. The catheters were frequently flushed with physiological saline (without addition of heparin) and the first 5–10 ml of blood drawn were discarded before samples are taken for coagulation factor analysis.

Plasma samples. Blood was drawn into 1/10 volume of 0.13 M trisodium citrate. In order to prevent further *in vitro* action of Defibrase, 0.01 ml of anti-Bodrops serum was added per ml of citrated blood. The blood was centrifuged at 3 000 g for 30 min at room temperature. The plasma was collected and kept frozen at -20°C until analyzed. Plasma samples to be used for factor VIII assay were kept at -40°C .

Serum samples. Blood was drawn into glass test tubes containing 8 mg of ϵ -aminocaproic acid (EACA) per ml drawn. The blood was left to clot at room temperature for 4 hours before centrifuging for 15 min at 1 000 g. The serum was collected and kept frozen at -20°C until use.

Fibrinogen determinations were performed by a micro method according to Bergström et al. (7). Interference of FDP with the fibrinogen determinations was studied as earlier described (20) and found to be negligible.

Fibrinogen determinations were also performed by a polymerization test on capillary blood according to Vermylen et al. (47).

Prothrombin was determined by a 2-stage method described by Nørre (36).

Factor V assays are performed according to Wolf (49).

Factor VIII was determined by a 1-stage method according to Nilsson et al. (35).

Factor X assays are performed according to Backlund et al. (3).

Factor XII was determined essentially according to the 1-stage method for factor VIII determinations (9) by using factor XII deficiency plasma as substrate. Used mainly as a screening method.

Factor XIII was determined by Lotz's fluorimetric amino incorporation method as modified by McDonald et al. (33).

Prothrombin and proconvertin are measured according to the method by Överin and Aas (38).

Normoxest and Thrombexin analyses as described by Överin and Strandell (39) and Överin (37), respectively.

Ethanol gelation test as performed according to Gödd and Abildgaard (23).

Estimation of soluble fibrin by N-terminal analysis. Blood for these analyses was collected in 1/10 volume of 0.13 M trisodium citrate containing 500 K₂ (Kallikrein inhibitory) units of Trasylol (Bayer, Leverkusen, West Germany) and 13 mg of EACA/ml. Fraction I (Cohn) was prepared from 3 ml plasma samples at final concentration of 12% ethanol and 0.015 M TAMC (non-

Fibrinogen
mg/ml

10.0

7.5

5.0

2.5



Fig. 1 Fibrinogen concentrations in Defibrase-treated patients. Zero time represents the time of the first infusion of Defibrase. \bullet = group A, \circ = group B and \square = group C patients (Table 1). Values for group C only before operation.

glycine methyl ester hydrochloride) (Sigma, St. Louis, USA) (1). The precipitate was dissolved in 1 ml of 0.3 M NaCl containing Trisylol and EACA in the above mentioned concentrations. After dialysis and adjustment of pH to 6.3 fraction I was further subjected to clotting by thrombin (20×3 h, 18 NIH units/ml). The clot was removed and rinsed in physiological saline which was added to the clot supernatant. Fraction I and the clot supernatant from fraction I were subjected to N-terminal analysis after lyophilization and dissolving in 10 M urea in 0.15 M TRIS-HCl buffer pH 9.5 glycine in the clotable portion of fraction I was calculated by subtracting glycine in the clot supernatant from the total amount of glycine in fraction I (1).

Analysis of N-terminal amino acids was performed according to the Edman procedure, as described for fibrinogen, using radioactive phenylisothiocyanate- 35 S (Radiochemical Centre, Amersham, Great Britain) (1, 16, 27). When samples were applied for descending paper chromatography in solvent III (45), carrier solution containing PTH derivatives of glycine, aspartic acid, alanine and tyrosine was added to each sample. Paper strips were examined in UV light and the spots with the same Rf values as glycine and tyrosine were cut out. The paper pieces were eluted in 2.0 ml 95% ethanol for 1 hour at room temperature before counting in Beckman liquid scintillator.

Fibrin(ogen) degradation products (FDP) are determined by the hemagglutination inhibition test described by Mersley *et al.* (24) (FDP-kit, Wellcome Reagent Ltd, Beckenham, Kent, Great Britain).

Immunoelectrophoresis was performed essentially according to Schneider (20, 43).

Spontaneous fibrinolytic activity of exogenous precipitates was measured on fibrin plates according to Ygge (50).

Fibrinogen and α_2 -macroglobulin are determined by the radial immunodiffusion method described by Mancini *et al.* (31).

Antithrombin III was determined by the Miacia technique as modified by Fagard and Abildgaard (22).

Determination of heparin neutralizing activity in patient serum. To 0.2 ml of normal plasma was added 0.1 ml of dilution of one part Defibrase and two parts patient serum or normal serum and the clotting times were recorded. When determined in 10 normal individual sera the average clotting time as 39.2 ± 1.4 sec.

RESULTS

Fibrinogen

Fibrinogen levels. An initial dose of 50 μ l Defibrase/kg \pm wt. was followed by a rapid depletion of fibrinogen though the rate and the extent of defibrination varied considerably (Fig. 1). The maximum amount of fibrinogen was removed by the initial dose within about 18 hours (Fig. 2).

In most cases the fibrinogen concentration was kept below 1.0 mg/ml during the period of treatment, but in order to keep the fibrinogen concentration at this level the standard dose of 50 μ l Defibrase/kg b.wt./day had to be increased in most patients (Table 1).

After cessation of Defibrase therapy there was an immediate rise in the fibrinogen concentration (Fig. 3). In most patients it rose to about 50% of the initial value within 4 days, but only one pa-

Table I. *Defibrase-treated patients. Duration of treatment, doses and fibrinogen values*

Group ^a	Pat. no.	Days of treatm.	Total no of infusions	Dose of Defibrase (minimum/maximum)		Fibrinogen (mg/ml)		
				μ l/infusion	μ l/day	Before treatm.	Mean during treatm.	Range
A	1	10	10	15/25	15/25	5.6	1.5	0.6-4.2
	2	6	6	10/20	10/20	3.5	1.0	0.6-1.7
	3	9	9	35/40	35/40	3.0	0.4	0.2-0.7
	4	9	9	35/40	35/40	3.8	0.8	0.2-1.1
	6	10	10	20/30	20/30	5.2	1.7	1.3-3.2
	7	10	10	33/40	33/40	3.2	0.6	0.5-1.3
	9	7	7	20/35	20/35	4.7 ^b	1.5 ^b	1.1-2.9 ^b
B	16	6	6	10/20	10/20	5.2	1.1	0.4-2.8
	17	10	10	10	10	2.3	0.8	0.5-1.2
	5	14	18	25/60	50/71	11.0	1.1	0-4.8
	8	10	10	50	50	4.1	0.5	0.1-1.5
	10	7	8	38/60	40/75	4.8	0.9	0-2.5
	12	10	10	50/60	50/60	5.4	0.9	0.6-1.0
	13	9	9	50/60	50/60	3.2	0.8	0.5-1.3
C	15	10	10	50	50	3.2	0.6	0-2.3
	18	10	11	20/70	40/70	9.4	0.7	0.5-0.9
	19	10	10	50/60	50/60	2.7	0.4	0-0.7
	20	10	10	50	50	3.6	0.5	0.3-0.7
	21	6	6	50	50	1.2	0.4	0-0.9
	22	15	21	35/70	50/100	7.5	1.0	0-3.1
	23	19	9	39/64	50/154	2.8	0.2	0-1.2
C	24	4	6	50	50/100	3.3	0.6	0.4-1.0
	25	5	7	50/55	50/110	3.4	0.5	0-0.9
	26	11	24	45/50	50/157	3.6	0.5	0-1.4
	27	17	38	44/57	50/138	4.6	0.7	0-1.8
	28	4	5	16/50	47/50	3.5	0.7	0-1.1
	29	14	14	50/61	50/61	2	0.7	0-1.8
	30	21	34	15/59	15/88	2	0.7	0-1.8
	31	5	5	50	50	6.7	0.8	0.5-1.2
	32	9	9	50/62	50/62	5.2	1.0	0-3.1
	33	7	7	50	50	3.2	0.7	0.3-0.8
	34	8	15	12/30	24/150	3.8	0.5	0-1.2
	35	12	30	14/70	29/129	7.3	0.4	0-1.0

A=low doses, B=high doses, C=high doses and surgical treatment

^b Determinations only by the polymerization method.

patient out of 13 represented in Fig. 3 reached the initial fibrinogen concentration within 4 days. Five patients were observed for 5-8 days after treatment but did not reach their initial fibrinogen values within that time.

Dose-response relationship Nine of 18 patients in whom no surgery was undertaken were given small doses of Defibrase 10-40 μ l/kg b.wt./day. The fibrinogen depletion was unsatisfactory in this group of patients (Table I, group A). The remaining patients were given 50-76 μ l Defibrase/kg b.wt./day and the average fibrinogen concentrations exceeded 0.7 mg/ml in four of these patients (Table I group B).

In 15 patients treated with Defibrase in con-

nection with surgery various doses of Defibrase (50-154 μ l/kg b.wt./day) were given in order to obtain a fibrinogen level giving adequate hemostasis and yet a good anticoagulant effect. The average fibrinogen concentrations exceeded 0.7 mg/ml in only 3 of these patients (Table I, group C).

Comparison of two methods for the estimation of fibrinogen concentrations during Defibrase treatment Values for fibrinogen determined by the polymerization method (7) were frequently observed to be lower than those by the syneresis method (7) even if the difference due to the fact that the former values were for whole blood and the latter for plasma was taken into consideration.



Fig. 2 Grams of fibrinogen removed during 24 hours after the initial dose of Defibrase. The total amount of circulating fibrinogen was roughly estimated by calculating the plasma volume as 4.0% and 3.5% of the body weight for men and women, respectively (Handbook of physiology Circulation, vol. 1 ed. W. F. Hamilton, American Physiological Society 1962). The values given represent the amount of fibrinogen removed per 10 μ l Defibrase given as the initial dose. Thirty-two calculations were performed on fibrinogen values obtained from 26 patients belonging to all three groups (Table 1). Horizontal bars represent mean values for the intervals indicated by the vertical dotted lines.

At plasma fibrinogen concentrations below 0.6 mg/ml (determined by the syneresis method) the fibrinogen concentration was not measurable by the polymerization method in any of the samples. At plasma fibrinogen concentrations between 0.6–



Fig. 3 Fibrinogen values for 13 patients after termination of Defibrase treatment. Zero time represents the time of the last Defibrase infusion. Symbols as in Fig. 1.

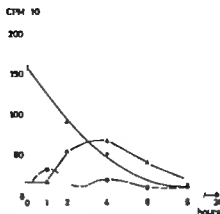


Fig. 4 Fibrinogen, soluble fibrin and FDP estimated by quantitative N-terminal amino acid analysis performed on fraction I obtained from successive plasma samples from patient 31. Zero time represents the time of the first Defibrase infusion. \bullet = N-terminal tyrosine in fraction I representing fibrinogen. \circ = N-terminal glycine in the clottable portion of fraction I representing soluble fibrin. Δ = N-terminal glycine in the non-clottable portion of fraction I representing high molecular weight FDP.

1.5 mg/ml (determined by the syneresis method) the fibrinogen concentration was frequently unmeasurable by the polymerization method, i.e. at 0.6 mg/ml and 1.2 mg/ml the mean values for determinations by the polymerization method were 0.1 ($\sigma=0.3$ $n=8$) and 0.5 ($\sigma=0.4$ $n=12$) respectively.

Ethanol gelation test (test for fibrinemia)

During the first 12 hours of treatment the ethanol gelation test was positive. Occasionally positive tests were also observed during further treatment.

Soluble fibrin estimated by N-terminal amino acid analysis (Fig. 4)

The amount of N-terminal tyrosine representing fibrinogen present in fraction I, prepared from successive plasma samples, fell in parallel to the fibrinogen concentration as determined by the syneresis method.

During the first hours glycine appeared in the clottable portion of fraction I, apparently representing soluble fibrin. The ethanol gelation test was positive during this period.

The non-clottable portion of fraction I contained almost no tyrosine but did contain appreciable amounts of N-terminal glycine, probably re-

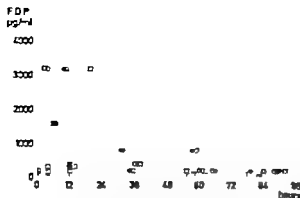


Fig. 5 FDP concentrations during Defibrase treatment obtained on successive serum samples from 16 patients. Zero time represents the time of the first Defibrase infusion. Symbols as in Fig. 1. Values for group C only before operation.

presenting partly proteolysed fibrinogen and fibrin high molecular weight FDP. A maximum was seen 4 hours after the Defibrase infusion. This was concomitant with an increase of FDP as measured by the Merskey test.

Fibrin(ogen) degradation products (FDP)

Serum samples taken 2 hours after the first Defibrase infusion and throughout the treatment period contained increased amounts of FDP (Fig. 5). The maximal concentration of FDP was found mostly in samples taken during the first 24 hours of treatment, though the amount of FDP varied



Fig. 6. Immunoelectrophoresis on serum sample obtained 6 hours after the initial dose of Defibrase (patient 31).

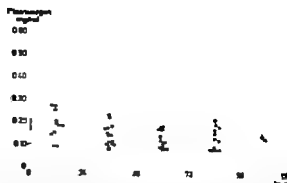


Fig. 7 Plasminogen concentrations during Defibrase treatment. Zero time represents the time of the first Defibrase infusion. Symbols as in Fig. 1. Values for group C only before operation.

considerably. Throughout the treatment period an increased amount of FDP was found in the serum (mostly 50–400 µg/ml). The immunoelectrophoretic pattern indicated the presence of early (Y products) as well as late (D and E products) FDP (Fig. 6).

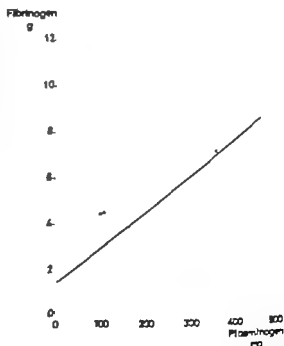


Fig. 8. Relation between the total decrease of fibrinogen and plasminogen after the first dose of Defibrase. Calculations were performed according to the method described in the legend to Fig. 2. The values given are based on 26 samples obtained from 19 patients from all three groups at various intervals 4–4 hours after the first Defibrase infusion.

Table II. Plasminogen and α_2 -macroglobulin concentrations during Defibrase treatment

Pat. no.	Plasminogen (mg/ml)				α_2 -macroglobulin (% of normal)			
	Before treatm.	during treatm.	Range	Mean	Before treatm.	during treatm.	Range	Mean
2	0.24	0.22	0.16-0.32	115	115	111	122	
3	0.35	0.26	0.21-0.28	58	58	55-62		
4	0.34	0.18	0.14-0.20	70	55	47-62		
5	0.36	0.15	0.04-0.23	145	121	90-172		
6	0.40	0.33	0.30-0.33	136	146	146-173		
7	0.29	0.13	0.08-0.16	80	74	65-80		
8	0.27	0.13	0.09-0.15	66	61	55-71		
9	0.41	0.27	0.22-0.31	124	119	100-140		
10	0.23	0.17	0.17-0.20	59	62	39-73		
12	0.33	0.15	0.13-0.19	139	76	67-96		
18	0.33	0.27	0.24-0.29	81	79	74-85		
17	0.30	0.21	0.18-0.27	94	94	74-115		
18	0.18	0.09	0.06-0.10	80	71	61-81		
20	0.27	0.17	0.13-0.20	67	70	67-74		

Spontaneous fibrinolytic activity

No increased fibrinolytic activity could be detected on fibrin plates during the treatment period.

Plasminogen

Within 24 hours after the first Defibrase infusion at 50 ml/kg b.wt. the plasminogen concentration had decreased in all patients (Fig. 7). The rate and the degree of plasminogen depletion, however, varied greatly. A correlation ($r=0.69$) could be found between the total amount of fibrinogen disappearing from the circulation during the first 24 hours of treatment and the total decrease of plasminogen within the same period (Fig. 8).

During further treatment the plasminogen concentration remained at a fairly constant level, though there was a considerable individual variation of the degree of plasminogen depletion (Table II). The mean values for plasminogen concentration during steady state of treatment was 28-94% (average 57%) of the initial concentrations. Also during this period the degree of plasminogen depletion was correlated to the degree of defibrination ($r=0.72$).

The initial plasminogen concentration was regained within 4 days after the last Defibrase injection in 5 of 12 patients (Fig. 9).

α_2 -macroglobulin (plasmin inhibitor)

The concentration of α_2 -macroglobulin was

Plasminogen 150
% of initial
value

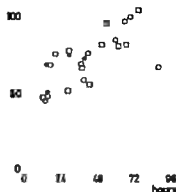


Fig. 9 Plasminogen values for 12 patients after termination of Defibrase treatment. Zero time represents the time of the last Defibrase infusion. Symbols as in Fig. 1

slightly decreased in most patients. A range concentrations were 94% (range 58-145%) and 87% (range 55-166%) of normal before and during Defibrase treatment, respectively (Table II). This decrease was not significant but correlation was found between the decrease of plasminogen concentrations and the decrease of α_2 -macroglobulin concentrations ($r=0.86$).

Fibrin stabilizing factor (factor XIII)

Factor XIII was determined in only 3 patients. Within 24-48 hours of the first Defibrase infusion there was a 50-60% decrease in the factor XIII value. One patient showed a further slow decrease to a final concentration of 25% of the initial value during treatment.

Coagulation factors II V VIII X and XII

No significant reduction of coagulation factors V, VIII and XII was observed during treatment, though the values obtained fluctuated, especially for factors V and VIII. Examples from 6 patients are given in Table III. Prothrombin-proconvertin tests were also mainly unchanged, as were the Normotest and factors II and X assays, until dicumarol treatment caused a decrease.

Antithrombin III

The concentration of antithrombin III was unchanged during Defibrase therapy in all but one

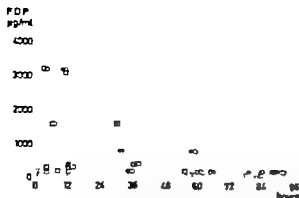


Fig. 5 FDP concentrations during Defibrase treatment obtained on successive serum samples from 16 patients. Zero time represents the time of the first Defibrase infusion. Symbols as in Fig. 1. Values for group C only before operation.

presenting partly proteolysed fibrinogen and fibrin high molecular weight FDP. A maximum was seen 4 hours after the Defibrase infusion. This was concomitant with an increase of FDP as measured by the Menkey test.

Fibrin(ogen) degradation products (FDP)

Serum samples taken 2 hours after the first Defibrase infusion and throughout the treatment period contained increased amounts of FDP (Fig. 5). The maximal concentration of FDP was found mostly in samples taken during the first 24 hours of treatment, though the amount of FDP varied



Fig. 6 Immunoelectrophoresis on serum sample obtained 6 hours after the initial dose of Defibrase (patient 31).

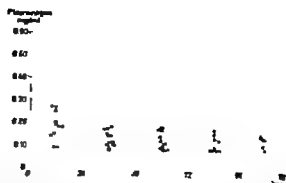


Fig. 7 Fibrinogen concentrations during Defibrase treatment. Zero time represents the time of the first Defibrase infusion. Symbols as in Fig. 1. Values for group C only before operation.

considerably. Throughout the treatment period an increased amount of FDP was found in the serum (mostly 50–400 µg/ml). The immunoelectrophoretic pattern indicated the presence of early (Y products) as well as late (D and E products) FDP (Fig. 6).

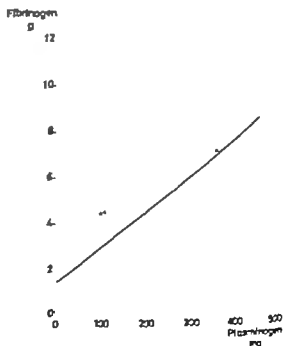


Fig. 8. Relation between the total decrease of fibrinogen and plasminogen after the first dose of Defibrase. Calculations were performed according to the method described in the legend to Fig. 2. The values given are based on 26 samples obtained from 19 patients from all three groups at various intervals 4–48 hours after the first Defibrase infusion.

Hemotocrit (% of normal)			Factor V (% of normal)			Factor VIII (% of normal)		
Before treatm.	Mean during treatm.	Range	Before treatm.	Mean during treatm.	Range	Before treatm.	Mean during treatm.	Range
92	83	70-100	70	106	83-120	128	131	101-238
—	103	90-162	115	82	62-153	90	69	49-104
54	69	60-88	110	139	105-172	120	105	68-153
80	123	95-140	165	168	120-203	228	149	99-203
68	120	56-160	113	107	69-205	—	—	—
46	78	48-107	85	179	64-480	109	252	96-1 000

teins might partly lead to a retention of the enzyme in the circulation and partly if the association is reversible, to a continuous liberation of free active enzyme.

The establishment of an adequate maintenance dose of Defibrase presented some problems. An initial dose of 10 μ l Defibrase/kg b.wt. was found to remove about 2 g fibrinogen within 18 hours. Since under normal conditions fibrinogen half life is about 100 hours (15) about 2 g fibrinogen is daily synthesized, 10 μ l Defibrase/kg b.wt./day would thus be expected to be an adequate maintenance dose. However according to our experience a maintenance dose of 100-150 μ l/kg b.wt./day would be required in most cases, preferably administered in 2-4 infusions per day. The reason for this discrepancy of the doses is not quite clear. Antibodies neutralizing the Defibrase activity could be one reason for the need for increased doses. Resistance to Arvin therapy and

an increased capacity of some patients sera to neutralize Arvin has been reported (41). Antigenicity of Defibrase is to be expected, since it is a glycoprotein having a molecular weight of about 40 000. Antibodies neutralizing Defibrase have been prepared from rabbit, goat and horse (46). The amount of enzyme administered during Defibrase therapy is of the order of 75-250 μ g/day (46). Clinically however no resistance to Defibrase was observed, but a sensitivity reaction to Defibrase cannot be excluded in one patient. According to the results presented here the capacity of plasma to neutralize Defibrase activity *in vitro* did not increase during treatment.

An increased rate of fibrinogen synthesis during Defibrase treatment seems to be the most probable explanation of the required doses of Defibrase. This hypothesis is partly supported by the fact that patients expected to have an increased fibrinogen synthesis, such as patients with acute leg thrombosis and operated patients, seemed to require higher doses of Defibrase than patients treated for central retinal vein thrombosis.

On the other hand, if the Defibrase-fibrinogen reaction is a first order reaction one would expect the substrate concentration to be the factor that limits the speed of the reaction at a given enzyme concentration. Consequently the velocity of the reaction would be comparatively lower at low fibrinogen concentrations and an increased need for Defibrase would be expected.

Large amounts of FDP appeared in the circulation during Defibrase treatment. A maximum concentration was mostly seen during the first 24 hours of treatment. Apparently a fibrinolysis

Table IV Defibrase-neutralizing effect of patient sera

Pat. no.	Days after initial defibrase infusion	Clotting time (sec) ^a
12	11	38.6
24	5	40.0
27	13	39.2
29	12	41.6
30	23	41.4
31	7	39.7
32	13	40.2
33	14	41.9

Average clotting time of 10 normal sera 39.2 ± 1.4 sec

was obtained secondary to fibrin formation, as earlier observed in dogs (20).

Similar results have also been reported for Arvin-treated patients. In spite of the appearance of FDP no increased plasma fibrinolytic activity could be demonstrated either in Defibrase or in Arvin-treated patients (6, 40, 44). Consequently the fibrinolytic process may take place intracellularly. Part of the degradation of fibrinogen or fibrin may be due to direct digestion by the enzymes (24-32) but the fact that the plasminogen concentration decreased during Defibrase as well as Arvin treatment (6, 44) indicates that plasmin is responsible for the fibrinolytic process. The decrease of α_2 -macroglobulin found in some Defibrase-treated patients further supports the assumption that plasmin is responsible for fibrin(ogen) degradation.

The concentration of antithrombin III was reduced in only one patient who in contrast to the other patients, had received heparin treatment for two days prior to the Defibrase treatment. A significant rise of antithrombin III concentration was seen during the Defibrase treatment. Abildgaard (2) has suggested an identity between antithrombin III and heparin co-factor (antithrombin II). Blombäck et al. (13) have further demonstrated a depletion of heparin co-factor during prolonged heparin treatment. The reported findings from this heparin and Defibrase-treated patient are probably due to a depletion of heparin co-factor during heparin treatment and a regeneration during Defibrase treatment.

The observed decrease of factor XIII during fibrinase treatment is in agreement with earlier *in vitro* findings that Defibrase activates factor XIII (9). The lowest value obtained was 25% of the initial value. According to findings in patients with congenital factor XIII deficiency an impaired hemostatic function is mostly not found at levels above 1% of normal factor XIII concentration (10). Prolonged and/or intensive Defibrase treatment may lead to such a depletion of factor XIII and should consequently be avoided, especially in patients with low pretreatment values (e.g. liver cirrhosis). In patients treated with Defibrase postoperatively a decreased factor XIII concentration may cause impaired wound healing, as would the hypofibrinogenemia as such (26). On the other hand the formation of stabilized fibrin clots during Defibrase treat-

ment in connection with surgical interventions may be of benefit for normal hemostasis.

The concentrations of other coagulation factors (II, V, VIII, X, XII) seemed to be unaffected during Defibrase treatment. The great variation for factors V and VIII was probably due to difficulties in arranging an appropriate blood sampling procedure. Neither does Defibrase treatment affect the platelet count (17, 18) or the platelet function (19). Similar results have been reported for Arvin treated patients (5, 6, 44).

The present investigation further confirms that Defibrase treatment mainly affects the level of circulating fibrinogen and that other probably secondary changes (i.e. occurrence of FDP, reduction of plasminogen, etc.) do not severely interfere with the capacity to maintain adequate hemostasis. No bleeding complications occurred in any patient treated for thrombotic disorders. Patients treated in connection with vascular surgery showed no excessive bleedings. From the hemostatic point of view Defibrase-induced hypofibrinogenemia appears to be as safe as any other kind of accepted anticoagulant treatment. In spite of the moderate hypofibrinogenemia obtained, an adequate anticoagulant effect was achieved in patients treated for leg thrombosis as judged from phlebographies performed after treatment.

ACKNOWLEDGEMENTS

This work was supported by grants from the S. Nels Medical Research Council, 19X 520, and from National Institutes of Health, HE 07379.

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THE HUMAN IMMUNE RESPONSE TO α -HAEMOCYANIN OF HELIX POMATIA

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Abstract. The immune response to α -haemocyanin of *Helix pomatia* (α -HPH) has been studied in 12 normal subjects. Humoral immunity was assessed by haemagglutination titres, cellular immunity by intracutaneous skin tests and the number of sensitized lymphocytes in the peripheral blood by *in vitro* lymphocyte stimulation. Immunological responsiveness to α -HPH (1.0 mg) was established with these parameters in all immunized subjects. The antibodies after primary immunization were nearly exclusively 2-mercaptoethanol-sensitive (2-MES) antibodies. Intracutaneous skin tests with 0.1 mg α -HPH after 28 days did not disturb the primary immune response, except in one subject who showed a rise in antibody titre although the antibodies remained 2-MES. Low levels of specific antibodies were present before immunization, probably so-called "natural" antibodies induced by exposure to cross-reacting antigens. After secondary immunization rapid rise in antibody titre occurred and the antibodies were nearly exclusively 2-MER (IgG). For the investigation of patients with immune deficiencies it will generally be sufficient to assess haemagglutination titres, delayed hypersensitivity and *in vitro* lymphocyte stimulation before and 3 weeks after immunization.

The purpose of this study was to discover whether α -*Helix pomatia* haemocyanin (α -HPH) was a useful test antigen for the investigation of the immune response of patients with immunological disorders like recurrent infections, auto-immune diseases and malignant tumours. α -HPH is a macromolecular respiratory protein from the Roman snail (*Helix pomatia*) which can easily be isolated while its physical and chemical properties are now well known. That is why we chose α -HPH for our investigations, the more so as it is known from the literature that related protein—keyhole limpet haemocyanin (KLH)—has good immunogenic properties in rats and rabbits (6, 14) and in man (3, 4, 13). The kinetics of the immune response to 1 mg α -HPH, subcutaneously were

studied in 12 normal subjects during one year. Immunological responsiveness was assessed by haemagglutination titres for humoral immunity, intracutaneous skin tests for cellular immunity while the reactivity of lymphocytes from the peripheral blood to antigen was studied in short-term *in vitro* lymphocyte cultures. One year after the primary immunization a secondary immunization with the same amount of α -HPH (1 mg) was given and the characteristics of this immune response were compared with those of the first.

In connection with the above mentioned practical clinical applicability of this system the technical details of antigen preparation, the immunological test systems and the results obtained will be described and discussed.

MATERIAL AND METHODS

α -Helix pomatia haemocyanin

Roman snails can easily be obtained in many countries of Europe; in the Netherlands they are collected in the southern part (Zuid-Limburg).

Pure α -haemocyanin was prepared in the Department of Biochemistry of the University of Groningen by the method described by Koolings et al. (10) and Huiswegh et al. (9). Professor M. Elzuber and Mr R. van Driel made the haemocyanin preparation used in this investigation available to us. The haemolymph, obtained by puncturing the heart of the snail, was centrifuged for 10 min at 10 000 g to remove debris. The clear supernatant was diluted with an equal volume of 0.4 M potassium acetate (pH 5.3), and the haemocyanin was precipitated by addition of an equal volume of saturated $(\text{NH}_4)_2\text{SO}_4$. After centrifuging for 10 min at 10 000 g the pale blue supernatant was discarded and the sediment was dissolved in 0.1 M potassium acetate (pH 5.7). This solution (total haemocyanin) contains a mixture of β haemocyanin, which can be distinguished by their behaviour in 1 M NaCl.

Table 1 Total haemagglutination titres (reciprocal) after primary immunization

The antibodies were 2 MES in all subjects except no. 7 who had low and transient 2-MER antibody titre. n.d. = not determined

Subject no.	Days after primary immunization									
	0	4	7	14	21	28	60	90	180	360
1	16	32	128	512	512	512	256	256	256	8
2	16	64	512	2048	2048	1024	512	256	128	16
3	16	256	512	2048	512	1024	1024	1024	256	64
4	4	4	128	128	128	64	32	32	32	n.d.
5	2	2	32	64	128	32	32	16	16	d.
6	16	64	128	512	256	256	128	128	128	16
7	16	16	512	2048	2048	1024	1024	512	256	128
7	Neg.	Neg.	16	16	64	64	32	Neg.	Neg.	Neg.
8	4	16	16	256	128	128	128	128	64	32
9	4	16	64	128	128	64	32	32	32	32
10	2	16	64	64	64	64	32	32	32	32
11	8	8	64	64	128	128	128	128	64	16
12	2	16	16	16	32	32	128	128	64	16
Mean	4/8	32	64/128	256	256	128/256	128	128	64	32

At pH 5.7 one component (α -haemocytin) is completely dissociated into 60 S particles by 1 M NaCl, while the other component (β -haemocytin) is not dissociated. They can then be separated by ultracentrifugation. α -HPII was stored at -70°C after lyophilization with sucrose (weight ratio sucrose/protein 2.75/0). For use it was dissolved in phosphate-buffered saline (PBS, pH 7.2) and, after dialysing against the same buffer, was sterilized by filtration through Milipore filter (0.45 μ). N antibiotics were added. After filtration the preparation contained 80% associated α -HPII (mol. wt. 9.10^5) and 20% halves. The protein concentration was determined routinely from the absorbance at 278 nm after adjusting pH to 9.2.

Immunization and skin testing

Twelve normal subjects, all members of the medical staff, ranging in age from 30 to 35 years, were immunized with 1.0 mg α -HPII subcutaneously in the deltoid region. Serial boosters before immunization intracutaneous skin tests with 0.10 mg α -HPII were done to exclude allergic reactions. There were all negative. Only 5 of these 12 subjects were skin-tested 28 days after immunization, because it was thought that reintroduction of antigen might disturb the primary immune response. One year after the first immunization a booster dose of 1.0 mg α -HPII was given subcutaneously to 10 of the 12 subjects.

Skin testing was performed with 0.10 mg α -HPII in 0.05 ml saline intracutaneously on the volar side of the forearm. The diameter of the induration area was measured at 24 and 48 hours. An area of induration larger than 5 mm after 4 or 48 hours was considered as a positive reaction.

Haemagglutination titres

Haemagglutination titres were determined by modified passive haemagglutination technique of Stavitsky (12).

Instead of sheep erythrocytes human O Rh+ve negative erythrocytes were used.

Erythrocytes, washed 10 times in PBS (pH 7.2), on mixed with an equal volume of a double saline and solution (0.005%), washed again and made up to a 4% solution in PBS (pH 7.2). Equal volumes of the erythrocyte suspension and α -HPII 0.8 mg/ml are incubated at room temperature for 30 min. The α -HPII-coated erythrocytes were subsequently washed 4 times in PBS, once in PBS (th 5% bovine serum albumin, and finally resuspended in a 4% suspension in PBS.

Control treated erythrocyte suspensions lacking α -HPII were prepared simultaneously. The sera to be tested were heated for 30 min at 56°C . Thereafter double dilutions were prepared. Both the α -HPII coated and the control erythrocyte suspensions are mixed with equal volumes of the serum dilutions on a haemagglutination plate and shaken during 10 min. Haemagglutination is determined macroscopically afterwards. The results were expressed as series of the reciprocal of the highest dilution of each serum which resulted in haemagglutination.

For each serum the total antibody titre and the titre after 4 hours treatment of the serum with 2-mercaptoethanol (2-ME) (0.1 M), the 2-MER antibody titre, are determined, the latter reflecting mainly the IgG antibody titre (5). The 2-mercaptoethanol-sensitive (2-MES) antibody titre, reflecting the macroglobulin fraction (IgM), can be determined by subtraction. The distinction between IgM and IgG on the basis of sensitivity to ME is not always reliable, however (13).

Lymphocyte cultures

Peripheral blood was immediately defibrinated in sterile flasks with 2 mm glass beads. For sedimentation 1.4 volume of methylcellulose 1% (Methocel Primavisc Grade 14 CPS, Dow Chem. Intern. Co.) is added and the blood incubated for 30 min at 37°C . The leucocyte-rich serum methylcellulose mixture collected, the cells

Table II. Total and 2 MER antibody titres (reciprocal) after secondary immunization

n.d. = not determined

Subject no.	Days after secondary immunization					
	0		14		21	
	Total	2 MER	Total	2 MER	Total	2-MER
1	8	Neg.	1 024	1 024	1 024	1 024
2	16	Neg.	2 048	2 048	2 048	2 048
3	64	Neg.	2 048	2 048	2 048	2 048
4	d.	—	—	—	—	—
5	n.d.	—	—	—	—	—
6	18	Neg.	2 048	2 048	1 024	1 024
7	128	Neg.	1 024	512	256	128
8	32	Neg.	2 048	2 048	512	512
9	32	Neg.	1 024	256	512	256
10	32	Neg.	1 024	1 024	512	512
11	18	Neg.	1 024	1 024	512	512
12	16	Neg.	1 024	1 024	2 048	2 048
Mean	32	Neg.	1 024/2 048	1 024	1 024	512/1 024

were washed twice in PBS and the WBC and differential count determined.

Cultures were set up in tightly closed tubes 17 × 100 mm (Falcon plastic). The culture volume, consistent at 5 ml in all cultures, adjusted to contain 1.10^6 lymphocytes/ml, as made of 20% autologous serum and 80% TC medium 199 (Difco Laboratories, Detroit, Michigan, USA) supplemented with penicillin 100 IU/ml and streptomycin 100 µg/ml. For each subject triplicates were made of unstimulated control cultures, cultures stimulated with phytohemagglutinin (PHA, Biorad, Wellesboro) 0.05 ml/culture, and cultures stimulated with α -HPH in the following amounts initially 0.005, 0.015, 0.030, 0.100 µg/ml of culture, later on only with 0.015 µg α -HPH/ml. Cultures were incubated at 37°C for 5 days, except PHA-stimulated cultures, which were incubated for 3 days. The medium was incubated in a standard incubator. pH correction was done with tris-HCl buffer (0.1 M, pH 6.8) if necessary. Blast transformation of the lymphocytes is determined by morphological evaluation of May-Grunwald-Giemsa stained cytocentrifuge preparations (1 000 lymphoid cells counted), and measured by the [14 C]-thymidine incorporation into DNA. Twenty-four hours before harvesting 0.6 µCi [14 C]-thymidine (Radiochemical Centre, Amersham), with specific activity of 58 mCi/mM, was added to the cultures.

At harvesting the cultures were washed once with cold PBS (4°C), once with acetic acid 2%, precipitated twice with 5% trichloroacetic acid, washed once in methanol and the acid-insoluble radioactivity was processed for liquid scintillation counting in standard manner. Quenching corrections were made. [14 C]-thymidine incorporation was expressed as ratio of stimulation:

[14 C]-thymidine uptake in d/min

in α -HPH-stimulated cultures

[14 C]-thymidine uptake in d/min in unstimulated cultures

Morphologically determined blast transformation was expressed as percentage of total lymphoid cells (percentage blast cells in unstimulated cultures subtracted).

RESULTS

Antibody formation

The results of the antibody determinations are summarized in Table I. A rise in antibody titre can be seen in 8 subjects after 4 days, while it was found in each subject after 7 days. Peak antibody titres occurred 2 and 3 weeks after immunization. Hereafter a plateau developed of a lower titre, which remained during 3 months or longer.

An important immunochemical property of the antibodies of nearly all subjects was the sensitivity to 2 ME. Only one subject (no. 7) produced also 2-MER antibodies. From Table I it can be seen that in all subjects antibodies existed before immunization, although in low titre. Probably this was not caused by specific haemagglutination, because erythrocytes coated with haemocyanin did not agglutinate in saline and tanned erythrocytes without haemocyanin showed no agglutination with these sera either. Moreover it could be demonstrated that the haemagglutination titres of all these preimmunization sera became negative after treatment with 2-ME, or after preincubation with an equal amount of an antigen solution (1.6 mg α -HPH/ml during 60 min at room temperature).

Table III Intracutaneous skin tests (0.1 mg α -HPH) 28 days after immunization

Subject no.	Delayed type response (mm ² induration)	
	24 h	48 h
8	16 15	12 12
9	20 20	neg.
10	16 16	18 18
11	15 15	20 15
12	12 18	15 16

In the last 5 subjects intracutaneous skin tests with 0.1 mg α -HPH were performed 28 days after the primary immunization. As will be seen from Table I there was no rise in antibody titre, except in subject 12, and 2 MER antibody production did not occur. On the contrary a significant rise in antibody titres occurred after subcutaneous administration of a higher antigen dose (1 mg) given one year later. The results are shown in Table II. All subjects produced high titres of antibodies, which nearly all were 2 MER.

Delayed type hypersensitivity

Intracutaneous skin tests with 0.1 mg α -HPH were performed just before immunization in all subjects and 28 days after immunization in the last 5 subjects. Before immunization neither immediate nor delayed type hypersensitivity reactions were found. At 28 days after immunization

there was a delayed type hypersensitivity reaction in all 5 subjects (Table III). Immediate type response being negative in all 5. The mean diameter of the induration area was 16 \times 16 mm. Histological confirmation of the delayed type hypersensitivity reaction to α -HPH was obtained in one subject, the biopsy showing infiltration of lymphocytes and macrophages.

Lymphocyte cultures

In vitro lymphocyte stimulation by α -HPH became obvious in all 12 subjects after immunization. The ratio of stimulation as shown in Table IV was less than 2.0 (considered negative) before immunization in 11 subjects, 2.5 in one subject (no. 2). In six subjects there was a positive response 7 days after immunization. There was a positive response in all tested subjects 14, 21 and 28 days after immunization. As can be seen in Table IV the high mean ratio of stimulation 7 days after immunization is due to two very high ratios of stimulation (nos. 9 and 10). In 7 subjects there was still a positive response 3 months after immunization. Morphologically determined blast transformation of the lymphocytes (Table V) showed a positive response 7 days after immunization in 7 subjects. All tested subjects showed blast transformation 21 days after immunization and all but one 28 days after.

Three weeks after the secondary immunization higher in vitro lymphocyte stimulation than 2 or 3 weeks after the primary immunization occurred

Table IV In vitro lymphocyte stimulation by α -HPH reflected as ratio of stimulation. [¹⁴C]-thymidine uptake in dilution in culture with α -HPH

[¹⁴C]-thymidine uptake in dilution in culture without α -HPH

Subject no.	Days after primary immunization									21 days after secondary immunization
	0	4	7	14	21	28	60	90	180	
1	0.9					4.0	9.0	2.2	7.5	42.3
2	2.5	2.3	15.2	7.1	24.4		9.5	2.3	1.1	23.4
3	0.3					18.0	10.6	4.3	2.1	58.0
4	0.9					27.0	16.0	6	2.3	—
5	0.6		2.1	2.6	15.3		7.2	1.2		—
6	1.2	1.3	3.2	2.4	6.8		3.7	3.0		20.5
7	1.2	1.0	0.8	6.9		3.6	1.5	2.5	1.0	9.5
8	1.0	2.7	1.3	2.8	4.8	31.1	2.0	1.8		24.2
9	1.0	0.9	50.1	2.3	3.0	18.0		1.2		3.9
10	0.8	1.0	104.4	8.2		3.6		5.4		37.8
11	0.9	1.3	9.6	2.4		4.6		0.9		2.2
12	1.1	0.9	1.9	2.8		2.3		1.9		5.2
Mean	1.1	1.4	20.9	3.9	10.8	12.5	7.4	2.4	8	22.7

Table V *In vitro* lymphocyte stimulation by α -HPH reflected as blast cell percentage (blast cell percentage in cultures without HPH subtracted)

Subject no.	Days after immunization								
	0	4	7	11	21	28	60	90	180
1	0.1	1.6	3.0		5.0	17.3	2.7	10.3	6.3
2	0.5	0.2	5.0	3.8	6.4			6.3	
3	0.0	1.5	12.5		8.2	8.9	4.5	7.1	3.4
4	0.0	3.2	4.5		8.2	5.8	3.3	3.7	0.4
5	0.0			1.2	4.5				
6	0.2	0.3	1.2	1.1	11.5			1.7	
7	-0.3	0.0		1.6			5.1		
8	0.1	0.5	1.2	6.5	9.7	7.8		3.0	
9	0.4	-0.3	3.8	6.6	5.9	6.7			
10	-0.5	-0.2	14.0	19.3		10.4		4.0	
11	0.1	-0.5	2.3	2.4	2.5			1.0	
12	-0.6	1.1	0.8	9.9		1.6		1.8	
Mean	0.0	0.7	4.9	6.0	7.0	8.4	4.0	4.3	3.1

in 8 of the 10 tested subjects (Table IV) while lymphocyte stimulation was absent in nearly all subjects 180 days after primary immunization.

Non-antigenic stimulation of the lymphocytes by PHA fluctuated between 80 000 and 250 000 d/min/culture in each subject during the period after immunization. We observed the same fluctuations in normal, non-immunized subjects.

The optimal antigen concentration for stimulation of the lymphocytes *in vitro* was studied in the first three immunized subjects. Concentrations varying from 0.005 to 0.030 mg α -HPH/ml culture medium induced an optimal response. There was a toxic effect of 0.100 mg α -HPH/ml in the lymphocyte cultures. Later on only a concentration of 0.015 mg α -HPH/ml culture medium was used.

DISCUSSION

From our results it can be concluded that α -HPH has good immunogenic properties in man.

Immunological responsiveness to α -HPH, as evaluated by haemagglutination titres, intracutaneous skin tests and *in vitro* lymphocyte stimulation, was clearly established in all immunized subjects. For the evaluation of immunological reactivity in patients it is important to have available an antigen with which there has been no previous contact. α -HPH seems to come up to this requirement because intracutaneous skin tests and lymphocyte stimulation tests were negative before immunization in nearly all subjects. Low

antibody titres were found before immunization, however.

Swanson and Schwartz (13) and Curtis et al. (3, 4) used KLH for investigation of immunological reactivity in man. Their results correspond to ours in general, both humoral and cellular immunity could also be induced by KLH and the kinetics of the immune response are comparable. They did not find antibodies before immunization, however. This discrepancy may be caused by differences in the performance of the passive haemagglutination test, such as a lower antigen coating of the erythrocytes, which makes the method less sensitive. An argument for this is the demonstration of synthesis of specific antibodies to KLH by lymph node cells from non-immunized rabbits by Folds and Stavitsky (8).

Specific antibodies before immunization, the so-called "natural" antibodies, have been observed for many particulate and soluble antigens (2) have been demonstrated by several techniques (7, 11) and may be IgM and/or IgG globulins (12). It is assumed that such "natural" antibodies are induced by exposure to cross-reacting antigens (2). It must be noticed that such cross-reacting antigens obviously give a low grade of immunity to α -HPH because the antibody titres were low (1/2-1/16) while the skin tests were negative and *in vitro* lymphocyte stimulation was absent, except in one subject.

After secondary immunization one year later a rapid rise in antibody titre occurred - the antibodies were nearly excluded.

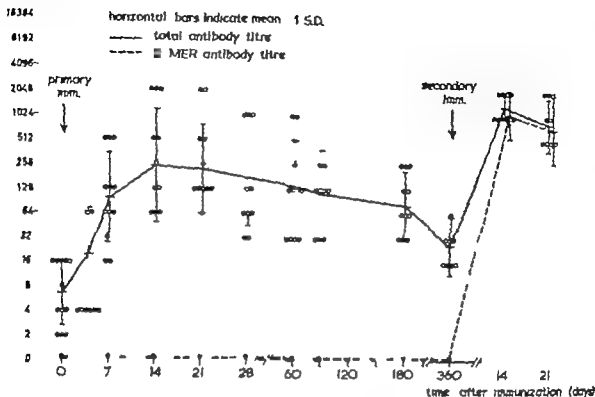
haemagglutination
titre

Fig. 7 Total and 2-MER antibody titres after primary and secondary immunization.

be seen in Fig. 1. These are characteristics of the so-called anamnestic or secondary immune response. Besides this distinctive humoral reaction there was an increase of *in vitro* lymphocyte reactivity to antigen compared with the primary response in 8 of the 10 subjects.

Another difference between the results of Curtis et al. (4) with KLH and our results with α -HPH was the antibody class in the primary response. KLH induced both 2-MES and 2-MER antibodies, α -HPH only 2-MES antibodies. The difference may be due to the different immunogenic properties of related haemocyanins (1), but the possibility of its being due to recurrent re-introduction of antigen by skin tests as performed by Curtis et al. (4) and Swanson and Schwartz (13) cannot be ruled out. We could show no influence of skin tests except for an increase in 2-MES antibody titre in one subject, but performed only one skin test late in the immune response.

From the kinetics of the immune response after primary immunization it can be concluded that,

for practical applicability in the investigation of immune disorders in patients, it will be sufficient to assess haemagglutination titres, delayed hyper sensitivity and *in vitro* lymphocyte stimulation before and 3 weeks after immunization. It must be stated that no unpleasant reactions to primary immunization with α -HPH were found. Perhaps it is important for some selected patients to immunize a second time with 1 mg α -HPH in order to study the capacity of 2-MER antibody production.

ACKNOWLEDGEMENT

This work was supported by a grant from the Netherlands Organization for the Advancement of Pure Research Z.W.O. (no. 91007).

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THE RELATIONSHIP BETWEEN SODIUM FLUXES AND ELECTRICAL POTENTIALS ACROSS THE NORMAL AND INFLAMED HUMAN RECTAL WALL IN VIVO

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Abstract. The normal human rectum generates a substantial transmural potential difference (PD), which in vivo is almost unaffected by the intraluminal ionic composition. The relationship between sodium transport and electrical PD across the intact human rectal wall has been studied in three healthy subjects and in eight patients to elucidate the significance of sodium movements in decreasing PD in ulcerative colitis (UC) and Crohn's disease. A linear relationship was found between the log of the ratio for bidirectional sodium fluxes and the transmural PD with a ratio of unity at zero PD. As significant increase in absorption and only moderate but significant decrease in secretion occurred in the patients, the initial changes of sodium transport and PD in inflammatory bowel disease are explained in terms of altered permeability characteristics of the rectal mucosa, probably followed by damage to the active sodium pump. Accordingly the permeability coefficient of the inflamed mucosa was estimated to be 10^{-6} to 10^{-8} cm sec $^{-1}$ while the diffusional sodium permeability coefficient of normal colonic mucosa was of the order $5 \cdot 10^{-6}$ cm sec $^{-1}$.

The normal human rectum generates a substantial transmural potential difference (PD) considered to be caused by active transport of sodium across the epithelium and effected by an energy-consuming sodium pump, situated at the serosal border of the epithelial cells (7, 17, 19). In ulcerative colitis (UC) this PD is significantly reduced (15), and it has been demonstrated that the polarization of the mucosa is never reversed (24), but in severe cases it may be abolished. The present investigation was carried out to elucidate the significance of sodium movements in decreasing PD in UC and Crohn's disease.

Although the interior of the mucosal cells is

probably negative to the luminal as well as the serosal surface of the cells—as has been shown in rat colon (18)—the epithelium as a whole may be considered an ohmic resistor so that for a given current the resulting transmural PD is determined by the resistance of the tissue to ionic flow. Sodium has proved to be the source of the electric current in the short-circuited frog skin (28), and seems also to account for the short-circuit current and the spontaneous transmural PD across the colon in several amphibians (4, 5), while a discrepancy in the current to flux ratio has been found in a study of isolated human colon (20).

Studies of the bioelectrical properties of colon in the intact organism are complicated by the fact that the short-circuit technique and a chemical inhibition of cellular metabolism cannot be applied in vivo and that the PD is practically unaffected by the luminal ionic composition in vivo (8). The decreased or abolished PD in diffuse UC (15, 24) however offers a possibility to investigate how the polarization of the rectal mucosa is correlated to the sodium transport.

The purpose of the present study was to quantify the relationship between the PD and the sodium transport across rectal mucosa by monitoring the effects of UC and Crohn's disease on the named parameters, and to give an estimation of the mucosal permeability in health as well as in disease.

MATERIAL AND METHODS

Patients and healthy subjects. To avoid contamination by stools in patients with diarrhoea, the rectal stump of six females and two males (aged 24-58), who had under

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gone complete colectomy for proximal disease 9 months to 6 years before the investigation, was performed *in situ*. Four patients had proctitis of the type associated with chronic UC, while the other four had biopsy criteria of Crohn's disease localized to the rectum, and/or segmental proximal disease as seen in Crohn's disease prior to operation. At the time of investigation seven of the patients had diffuse proctitis and belonged to grade II-III according to the classification of Baron et al. (1). One male (case 1) was in complete remission (grade 0) after local treatment with steroids. None of the patients studied was currently receiving treatment with corticosteroids or salazo-sulfapyridine. Sigmoidoscopy was performed, and biopsy specimens of the mucosa were taken on the day following the absorption study in order not to break the mucosal integrity. The macroscopic as well as the microscopic examination of mucosa revealed no gross lesions of the epithelial layer which seemed to be intact.

Observations were also made in three healthy volunteers (aged 25-76), one of whom was female.

Rectal absorption studies. The ratios of bidirectional sodium fluxes as well as the transmucosal PD in the rectum were determined simultaneously in the same individual.

Sodium transport was measured during constant perfusion of the rectum. A rubber tube (o.d. 0.3 cm) was introduced through a sigmoidoscope and left with its oral opening at the top of the isolated rectum, approximately 15 cm from the anus. Initially the volume of the rectum (45-65 ml) was assessed by measuring the amount of solution infused until fluid appeared from another rectal tube, the opening of which was placed 3 cm within the anus. Meanwhile the infusion rate was adjusted to 5 ml/min, and after 45 min of clamping it was usually possible to attain a steady state according to the criteria of Devroede and Phillips (9). The rectal effluent was then collected for analysis every 15 min during a 1.2-hour period. Finally as much fluid in the rectum as possible was removed by introducing air into the gut lumen, and the remaining intraluminal content plus 100 ml of the original test solution were

replaced at a rate of 5 ml/min to prolong the time contact between the solution and the absorbing surface. After 60 min 2-3 samples were collected from the rectum at 2-min intervals. In the healthy subjects a vertical balloon incorporated in a double-lumen tube isolated the rectum 15 cm oral to the anus by a modification of the method described by Devroede and Phillips (11). The test solution was infused just below the balloon, which was inflated with 50 ml water. If faecal contamination occurred, the experiment was considered a failure and excluded from the study. Otherwise samples were collected for analysis as described above. During the experiment the subject lay on his right side.

The test solutions contained varying concentrations of (A) NaCl in the molar ratio of 1:1, or (B) sodium, chloride, potassium, and bicarbonate in the ratio of 1.00:0.75:0.21:0.46 to stimulate ileal discharge (11). In solution A the resulting sodium and chloride concentrations ranged from 125 to 145 mEq/l. In solution B the concentrations of sodium ranged from 110 to 125 mEq/l, chloride from 80 to 95 mEq/l, potassium from 23 to

26 mEq/l, and bicarbonate from 50 to 58 mEq/l. No patient or control was tested more than once with each test solution by each of the techniques described above. The mean intraluminal concentration of sodium in the individual experiments is given in Table I. All solutions were made isotonic by adding urea and fructose, the absorption of which was determined for other purposes; 1.5 μ Ci 22 Na, 0.5 g neomycin, 2 mg Cycloheximide/l, and as non-absorbable marker substances tracer doses of 45 Cr-EDTA (Boehringer, Frankfurt am Main, Western Germany), 57 Co-vitamin B₁₂ (Radiochemical Centre, Amersham, England), and 51 I-PVP (Radiochemical Centre) were added. By Sephadex gel chromatography (Sephadex G-25 medium, Pharmacia, Uppsala, Sweden) 51 I-PVP was always checked for free radioactive iodide, which generally was less than 1% but on single occasion amounted to 8%. When batch proved to contain more than 0.5% of the activity as free iodide, preparation of the high molecular fraction of 51 I-PVP was made by gel chromatography and used instead (25).

Colonic absorption studies. For the gross estimates of mucosal permeability to health the across to mucus flux of sodium into the entire colon was determined, as this flux across the normal rectal mucosa was too small to be measured with reasonable accuracy. Two slightly different modifications (9, 23) of the colonic perfusion technique originally described by Levitan et al. (22) were used. The colon of nine healthy subjects (aged 16-47) was perfused at a constant rate from the caecum to the rectum with a 145 mM NaCl solution containing marker and test substances as described above. To test whether the across to mucus flux of sodium across the jejunal colonic wall is dependent on the transconcentration for the flux as is the case in the rat (6), the perfusion was repeated on three occasions on the following day using an isotonic mannitol solution instead. Six sequential 15-min samples were collected from the rectum for analysis. In seven subjects slow marker perfusion of the terminal ileum was carried out as described elsewhere (9, 23), permitting correction of the calculated absorption value for fasting ileal contents entering the colon.

Analytical procedures. All analyses were carried out in duplicate. Concentrations of sodium were measured by means of a flame photometer (Lustration Laboratory flame photometer Lexington, Mass. USA) using lithium as internal standard. Immediately after the experiment the 22 Na activity was measured twice in 3 ml samples from the perfusion solution, and from each 15-min period in shielded well type scintillation counter (Sodium iodide well, 3 inches; dual channel analyzer Model 45-23, Selttronik A/S, Copenhagen, Denmark). After at least 10 57 Co half-lives 57 Co, and 51 Cr activity was determined using the appropriate correction.

The rectal samples of the perfusion fluids were routinely checked for haemoglobin spectrophotometrically and for protein by means of an Alkabsorb.

Electrical measurements. The technique and the equipment for measuring the PD was identical to that described elsewhere (24), except that the exploring KCl salt bridge was thinner (i.d. 0.2 cm) and always introduced from the rectum blindly during the latter part of the infusion period for continuous registration (Servogor,

Table I. Sodium transport transmembrane potential difference and sodium flux ratio of the normal and inflamed rectum (mean \pm S.D.)

CD = Crohn's disease

Subject no.	Disease	Test solution	No. of flux periods measured	Sodium transport (μ Eq/min)			Mean N flames/Na plasma (μ Eq/l)	PD (mV)	Sodium flux ratio		
				Net ^b	Inscription	Exsorption			Observed	Calculated	Observed/calculated
<i>Normals</i>											
I	—	B	5	40 \pm 5	46 \pm 2	6 \pm 3	108/139	-38	7.7	0.18	43
		B ^a	3	39 \pm 1	71 \pm 5	12 \pm 4	108/139	-38	5.9	0.18	33
II	—	A	4	23 \pm 6	25 \pm 6	2 \pm 0.5	122/146	-42	13	0.17	77
		B	2	30	32	2	101/146	-42	16	0.14	114
III	—	B	3	27 \pm 7	29 \pm 8	2 \pm 0.5	118/143	-39	15	0.18	75
		B ^a	2	58	66	8	116/143	39	8.2	0.15	49
<i>Patient's remission</i>											
1	UC	A	3	20 \pm 4	23 \pm 6	3 \pm 2	125/138	-26	7.6	0.34	22
		A ^a	3	40 \pm 6	60 \pm 3	20 \pm 7	125/138	-26	5.0	0.34	22
<i>Patient's relapse</i>											
4	UC	B	4	-1 \pm 7	18 \pm 7	19 \pm 12	126/139	4	0.95	1.1	0.86
		B ^a	2	-19	48	67	134/139	+4	0.72	1.0	0.72
5	CD	A	5	8 \pm 7	15 \pm 4	7 \pm 4	142/143	-12	2.1	0.63	3.3
		A ^a	3	10 \pm 3	38 \pm 1	28 \pm 3	141/143	-12	1.4	0.62	3
6	CD	A	4	-13 \pm 4	20 \pm 3	35 \pm 4	134/148	-2	0.57	0.84	0.68
7	UC	A	7	-26 \pm 5	5 \pm 2	31 \pm 5	133/141	2	0.16	0.87	0.18
8	UC	A ^a	3	21 \pm 11	32 \pm 16	11 \pm 9	142/143	-3	2.9	0.88	3.3
9	CD	A	3	18 \pm 1	31 \pm 4	13 \pm 4	143/140	-16	2.4	0.55	4.4
		A ^a	3	65 \pm 14	93 \pm 13	28 \pm 4	143/140	-16	3.3	0.55	6.0
11	CD	B	3	6 \pm 5	18 \pm 7	12 \pm 4	114/139	3	1.5	0.92	1.6
		B ^a	3	11 \pm 4	28 \pm 5	17 \pm 5	114/139	3	1.6	0.92	1.7

100 ml standard solution was recirculated for 60 min. These fluxes were calculated on the basis of perfusion speed of 5 ml/min. Values paying regard to the fact that the standard solution was being recirculated.

Positive values denote absorption, negative values excretion.

Observed ratio calculated from inscription/exsorption, calculated ratio from Na flames/N plasma /antilog E(mV)/60.

Goetz Electro, Vienna) of the transmembrane PD at the top of the rectum. As the serosal surface is equipotential to blood (7), one of two balanced calomel half-cells was connected to the low impedance input of high impedance electrometer (PH-Meter 51 Radiometer Copenhagen) and to each of the foreruns via flowing 154 mM NaCl salt bridge inserted for security reasons. The other half-cell connected the high impedance input of the electrometer to the exploring salt bridge. At the end of the perfusion period the PD was measured throughout the rectum and the mean value of 3-5 measuring sites was determined. The difference between the maximum and the minimum values in the same individual is generally 1-2 mV but in one healthy subject

variance range of 5 mV was found. It was checked that the use of mannitol, fructose, and urea had no effects on the transmembrane PD either in patients or in healthy subjects.

No attempts were made to correct for the junction potentials due to simultaneous use of NaCl and KCl bridges, as almost identical NaCl solutions in the rectum and in the l. NaCl salt bridge give rise to junction potentials of equal size but with opposite signs. The junction potential between the l. isotonic sodium chloride

and the plasma is negligible—as discussed in detail by Dalmont (7)—and this small potential is of the same magnitude in healthy subjects and in patients.

Terminology and calculation. According to Code (3) the movement from the gut lumen to the blood is called absorption and the movement in the opposite direction exsorption. A net gain to the body is denoted absorption and net loss secretion.

Net changes of sodium are calculated from the concentration changes of ²²Na-PVP (22) is minimal, although significant amounts of ⁵¹Cr-EDTA and ⁵⁷Co-vitamin B₁₂ were always absorbed in the patients. Based on the disappearance rate of ²²Na, assuming zero concentration in the blood, absorption of sodium was found by modification (25) of the method of Vischer et al. (29). Hence the exsorption was taken to be the algebraic difference between inscription and net transfer of sodium.

In order to measure the actual absorption of the marker substances, the fractions of infused amounts of radioactivities recovered in the urine were determined. As ⁵¹Cr-EDTA is neither metabolized nor bound to proteins, and as the complex is excreted rapidly and quantitatively by the kidneys, the ⁵¹Cr activity in 48-hour urine for all practical purposes equals the amount of ⁵¹Cr-EDTA ab-

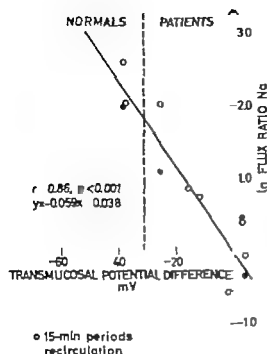


Fig. 1 Relationship between the transmucosal potential difference and the natural log of flux ratio for sodium in the human rectum.

absorbed (26). However not all ^{59}Co -vitamin B_{12} and only half the amount of ^{51}Cr -PVP absorbed will be cleared by the kidneys within this period. In supplementary investigations, therefore, the fractions of ^{51}Cr , ^{59}Co , and ^{51}Cr radioactivity which were excreted by the kidneys following an i.v. injection of known amounts of ^{51}Cr -PVP, ^{59}Co -vitamin B_{12} and ^{51}Cr -EDTA, were also determined. So the ^{51}Cr and ^{59}Co activities eliminated by the kidneys in 48 hours turned out to be 49.5 ± 0.6 (mean \pm S.E.M., $n=4$) and 91.9 ± 1.1 (mean \pm S.E.M., $n=4$) of the injected dose, respectively when the ^{51}Cr excretion was put at 100%. Consequently the urinary excretion of ^{51}Cr and ^{59}Co in the studies of rectal absorption were divided by 0.495 and 0.919 respectively to give 100% as well, viz. what had actually been absorbed. In all tests 5 mg unlabelled vitamin B_{12} was given intravenously 15 min prior to the investigation, and this dose was administered again 30 min later to ensure maximum renal glomerular filtration rate of radioactive vitamin B_{12} .

Statistical analysis was by Student's *t*-test. Calculations were facilitated by computer programmes.

RESULTS

No significant absorption of the volume markers ^{51}Cr -PVP, ^{59}Co -vitamin B_{12} and ^{51}Cr EDTA took place, since the total urinary radioactivities of these tracers corrected for differences in renal

Table II. Mean rates (\pm S.E.M.) of bidirectional sodium fluxes across the healthy human colon during perfusion with isotonic NaCl and mannitol solutions (perfusion rate 15 ml/min)

Perfusion solution	No. of subjects	No. of steady state collection periods	Na transport ($\mu\text{Eq}/\text{min}$)	
			Absorption	Excretion
NaCl (145 mM)	9	32	864 ± 160	234 ± 73
Mannitol isotonic	3	11	6 ± 1	41 ± 9

clearances (25) would account for 0.01–0.03 and 0.1–0.3% of the total activity infused into the rectum of controls and patients, respectively. The absorption of ^{51}Cr -PVP, ^{59}Co -vitamin B_{12} and ^{51}Cr EDTA was calculated to occur at the ratio of 1.0 ± 0.3 , 2.1 ± 0.2 , 3.1 ± 1.0 (mean \pm S.E.), respectively (25). Neither albumin nor haemoglobin was detected in the perfusate.

The relations between PD and sodium flux ratio at low PDs (seen only in disease) as well as at high PDs (seen normally) are shown in Fig. 1. The ordinate gives the logarithm of the flux ratio for the 15-min periods of perfusion or the period of recirculation, and the abscissa the mean PD for one subject. There is a linear relationship between the natural log flux ratio and PD, a ratio of 1 being obtained at zero PD.

Table I gives the mean values for the individual experiments based on 67 sampling periods in 11 individuals. The pattern of sodium transport in healthy subjects and in the only patient in clinical remission was characterized by net absorption of sodium and a flux ratio of 13–16. As the intraluminal sodium concentration was always less than that of plasma, and lumen always negative to blood, sodium was absorbed against an electrochemical gradient. In four out of seven patients in relapse sodium was absorbed, but the ratio of sodium fluxes did not exceed 3.3 and it was less than 1 in three patients in whom sodium was actually secreted into the rectal lumen. The net transfer of sodium was, in all patients, against a far smaller electrochemical gradient or even in the direction of the electrical as well as the chemical gradient.

The techniques of "steady state" perfusion and "recirculation" gave fundamentally identical al-

nes for the flux ratio. As the mean intraluminal Na concentration did not differ significantly in the two types of experiments, the rate of sodium transport should be independent of the total volume infused. However the values for sodium movements based on recirculation experiments were calculated from standard formulas without paying regard to the fact that the test solution was being recirculated and accordingly they came out to be two to three times those in experiments based on 15-min sampling periods (Table I). If it is taken into consideration that the same 100–150 ml test solution (rectal volume 0–50 ml + recirculation reservoir 100 ml) was infused during 60 min of recirculation, while (5 × 60 = 300 ml) were infused during the same period when the original technique of steady state perfusion was used, it will be seen that insorption-exsorption data in Table I (dividing recirculation values by 2) on an average are independent of the technique used. In normals a mean insorption of 32 $\mu\text{Eq/min}$ is obtained, and in patients not in remission one of 19 $\mu\text{Eq/min}$. Similarly for exsorption the means were 4 and 19 $\mu\text{Eq/min}$, respectively. In other words, in the patients, insorption decreased and exsorption increased significantly ($p < 0.01$) resulting in a reduced sodium flux ratio.

The variation in the transmural PD of the rectum at varying distances from the anus was generally less than 2 mV in the same subject. As the amplitude did not change when test solution A was replaced by solution B the mean value for both perfusion periods is given in Fig. 1 and Table I.

Table II shows the mean rates of bidirectional sodium fluxes measured during "steady state" perfusion of the entire colon. It is seen that the mean rate of exsorption is significantly reduced when the chemical gradient is increased (mannitol perfusion, $p < 0.01$).

DISCUSSION

In inflammatory disease the mucosal integrity may be broken, and there may be a significant exudation of fluid. So the possibility must be considered that significant secretion of sodium may be driven by hydrostatic pressure. The lack of albumin and haemoglobin in luminal fluid does not necessarily mitigate against increased ultra-

filtration, but indicates that capillary permeability was not grossly altered. However the fact that no significant absorption of the non-absorbable markers occurred seems to rule out the possibility that the epithelium was the seat of heavy lesions. Sigmoidoscopy as well as the microscopic appearance of mucosa confirmed this point of view as these examinations revealed no major defects or ulcerations of the epithelium. In conclusion the results of the present study, summarized in Fig. 1 suggest that the transmural PD reflects the capacity of the epithelium to transport sodium against an electrochemical gradient. The linear regression of the natural log flux ratio on PD shows a flux ratio of 1 when PD is zero indicating that sodium movements alone are responsible for the generation of the transmural PD of rectum.

If the sodium ion crossed the epithelium by simple diffusion only it should be possible to calculate the observed ratio of sodium fluxes from the equation developed by Ussing (27)

$$\frac{M}{N} = \frac{Na_i}{Na_o} \times \exp. \frac{z(\psi_i - \psi_o)F}{RT} \quad (1)$$

where M and N are the influx (insorption) and outflux (exsorption) of sodium, Na_i and Na_o the mean concentration of sodium in lumen and plasma, respectively; z the charge of the Na⁺ ion, $\psi_i - \psi_o$ the transmural PD, F , R , and T respectively are the Faraday constant, the gas constant, and the absolute temperature. The activity coefficients have been omitted, as the plasma and the luminal solutions were of approximately equal ionic strength.

The observed and calculated values of the flux ratio are compared in Table I. As expected there was a marked discrepancy in the normal individuals, which was more or less reduced in the patients. To explain this discrepancy an active transport of sodium must be taken into consideration as the relationship between the observed and calculated flux ratio applying to a true exchange-diffusion system should be more close to unity (see Table I). The observation of net N⁺ transport against an electrochemical gradient can be explained neither by exchange diffusion nor by interaction between sodium ions in the membrane (single-file diffusion). Other solute-solute couplings cannot be operative, as no other solutes than sodium and chloride are transported to a signifi-

cant extent from the gut lumen to the blood. Also the possibility of solvent drag can be excluded, as water absorption in the colon is accepted to be solute-linked and correlated to the net transfer of sodium (10).

It is clear from eq. 1 that the ratio of the opposed fluxes of sodium is equal to the ratio of the sodium concentration on either side of the epithelium when the PD is zero. Thus, any sodium transport may move down the difference in chemical potential in patients with a transmural PD around zero. The similarity between observed and calculated values in these patients does not rule out the possibility of an active sodium pump working in relation to a leaky membrane. An increased permeability of the inflamed mucosa has already been established by the significant absorption of the inert hydrophilic compound ^{51}Cr -EDTA in patients with UC (26). From the exsorption data in the patients with a PD around zero the passive permeability coefficient (P_{Na}) may roughly be estimated to be of the magnitude 10^{-2} – 10^{-3} cm sec $^{-1}$ which is a factor 10 to 10^3 greater than that obtained by Corran and Schwartz (6) and Edmonds (14) in the normal rat. This may mean that specific resistivity has approached zero, i.e. that ionic fluxes under hydrostatic and perhaps osmotic forces have essentially short-circuited the membrane. As regards patients with PDs between zero and normal values there is the possibility that they may in part at least, represent Na diffusion potentials (notwithstanding an electrogenic mucosa-to-serosa active transport). Thus, if the mucosa is inflamed or contains increased interstitial fluid, hydrostatic pressure may be increased, promoting bulk flow of water (and ions) into the lumen, particularly if increased hydraulic conductivity is present. Related to this is the possibility of artifact in the system with regard to isotopic measurements of unidirectional fluxes. Thus it might be assumed that sodium is actively transported into the lateral intercellular space where, on the standing gradient hypothesis (13), it exists at the serosa as an isotonic solution under normal conditions. If however bulk flow is occurring in the opposite direction (i.e. towards the lumen), isotopic sodium extruded into the lateral cell border might be swept back into the lumen. This could have at least three effects: (a) recirculation of isotope would generate falsely low mucosal-to-serosal fluxes, and therefore fal-

sely high serosal-to-mucosal fluxes with falsely low flux ratios, (b) it could create an osmotic gradient favourable to further bulk flow of water into the lumen, and (c) depending on relative mobilities of accompanying anions, it could generate a potential across the tissue, mucosa being slightly more positive as observed experimentally in some cases. For example the Na activity at the mucosal interface could be considerably higher than in the luminal bulk solution, and a Na diffusion potential could exist.

Under normal conditions with a substantial rectal PD the permeability of the human rectal mucosa is very low as indicated by the extremely low rates of exsorption in normals (Table I)—especially when it is taken into consideration that the sodium fluxes are probably accelerated by a carrier-mediated Na-Na exchange diffusion at the mucosal border of the epithelium (6). Although it may not seem reasonable to calculate Na permeability constants for normal colon and compare them to permeability constants for diseased rectum, perfusion of the entire colon was necessary to obtain values significantly different from zero. The results in Table II demonstrate that the exsorption is dependent on the transconcentration for the flux (6), so that the mucosal border of the epithelium seems to be almost impermeable to sodium when the mean concentration of the ion in the lumen is reduced to a few mEq/l. Assuming the colonic area to be 1000 cm 2 (2) the diffusional sodium permeability coefficient is thus less than 5×10^{-6} cm sec $^{-1}$ even without taking the electrical driving force into consideration. This value contrasts the result obtained in the diseased rectum, but is of the same order of magnitude as values given for rat colon (6, 14). Also Edmonds' (16) findings of a sodium flux ratio >1 as well as those of the present study suggest that rectum is considerably less permeable to this ion than other parts of the colon (12), which seems to complete the general pattern of increasing absorptive capacity for sodium, not only from the duodenum to the colon (21) but also from the caecum to the rectum. Under the circumstance of zero sodium concentration intraluminally the cell interior may become less negative relative to the serosal as well as to the mucosal border of the cells due to a decrease in sodium transport. However the resulting transmural PD need not decrease in the normal state as the PD across

the luminal border is abolished if the mucosal membrane is passively tight to all ions, or even reversed due to a chloride diffusion potential if non-selectively permeable to small ions. Accordingly *in vivo* experiments have demonstrated that the normal transmural PD is fairly independent of the intraluminal sodium and potassium concentrations (8).

The principal function of the human colon is conservation of salt and water (10), which gives the relationship between the flux ratio of sodium and PD clinical relevance, as a determination of the transmural PD proved to be a most simple and sensitive measurement of the functional integrity of the rectum in patients with inflammatory bowel disease.

ACKNOWLEDGMENTS

This study was supported by grants from the Danish Medical Research Council, the Novo Foundation, and the King Christian X Foundation.

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SERUM CALCIUM AND PHOSPHORUS IN PATIENTS TREATED WITH THIAZIDES AND FUROSEMIDE

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Abstract. Serum calcium and phosphorus have been determined in 36 patients treated with thiazide or furosemide diuretics for at least 3 months. In the group treated with thiazides serum calcium was significantly higher than in the control group. When serum calcium and phosphorus values were evaluated by linear discriminant function analysis, the group treated with thiazides differed significantly from its control group. No differences could be seen in the patients treated with furosemide as compared with their controls. Thiazide treatment should be considered in the differential diagnosis of disturbances in serum calcium and phosphorus metabolism.

Changes in serum electrolyte concentrations are the side-effects most commonly seen during diuretic treatment. To these disturbances have recently been added raised serum calcium values seen in some patients during thiazide treatment (1-5). In a case described by Duarte et al. (3), a transient increase in serum calcium concentration was found after the start of thiazide treatment, but despite continued thiazide administration normal serum calcium was recorded 2 weeks after the start of treatment.

The aim of the present investigation was to study whether changes in serum calcium or phosphorus concentrations can be seen in a randomly selected series of out-patients treated with thiazides. In view of the possibility that such effects are restricted to thiazides, patients who had received furosemide treatment were also included in the study.

MATERIAL AND METHODS

Case records of patients with the diagnosis hypertension or congestive heart failure were selected from the University Central Hospital, Helsinki, from the years 1968-69. An invitation to attend a clinical examination was

sent to the patients with the so mentioned diagnoses. Anamnestic data and current treatment were recorded, physical examination was made, and blood samples were obtained for chemical analyses. Serum calcium and phosphorus were analysed by routine clinical methods (5-14).

The patients studied were divided into four groups according to the treatment. 1) Hypertension treated with thiazides (chlorthalidose 500-1 000 mg/day hydrochlorothiazide 50-100 mg/day) (18 patients). 2) Hypertension, no diuretics given (14 patients). 3) Congestive heart failure treated with furosemide (40-80 mg/day) (18 patients). 4) Congestive heart failure, no diuretics given (12 patients). T. additional groups, hypertensive patients treated with furosemide and patients with congestive heart failure treated with thiazides, had to be omitted because only a few such patients were found.

RESULTS

The ages and sex ratios of the patients studied are shown in Table I. The age distribution of the treatment groups matched that of the controls. However the heart failure patients were older than the hypertensives.

A slightly higher mean serum calcium concentration was found in the group treated with thiazides than in the hypertension control group ($p < 0.05$). Mean serum phosphorus was lower and the calcium/phosphorus ratio higher in the patients treated with thiazides, although the differences were not significant ($0.1 > p > 0.05$ in both).

To gain further information on the relations of serum calcium and phosphorus in these patients, we subjected the calcium and phosphorus values to linear discriminant function analysis (4). The function obtained is that linear function of the two primary variables on which the overlap between the distribution of the two groups is mini-

Table 1. Effect of long-term thiazide and furosemide treatment on serum calcium and phosphorus

	No. of pts.	♂ ♀	Age (y)	Ca	P	Ca/P
Hypertension						
Treated with thiazides	18	5 13	49±11	10.86±0.59	2.91±0.41	3.81±0.57
Controls	14	5 9	50±12	10.46±0.50	3.15±0.54	3.41±0.57
			ns	$p<0.05$	$0.1>p>0.5$	$0.1>p>0.05$
Congestive heart failure						
Treated with furosemide	18	8 10	65±7	10.37±0.37	3.20±0.49	3.31±0.52
Controls	12	7 5	62±6	10.28±0.76	3.28±0.61	3.27±0.79
			ns	ns	ns	ns

mized. This is shown in Figs. 1 and 2, where the x-axis has been rotated into a new position, corresponding to the discriminant function, and a line (*L*) has been drawn parallel to it. The individual values of calcium and phosphorus are projected on this line. The cumulative distributions of the calcium and phosphorus values are shown above the line *L*. In the treatment and control groups the means±S.D. for the individual points on the discriminant function were calculated and are shown in the insets in Figs. 1 and 2. The differences between the means were evaluated using Student's *t*-test.

When the values for the hypertensive patients were submitted to this test, the group treated with thiazides differed significantly from the

controls (no thiazides) in respect both of calcium and phosphorus values ($p<0.025$).

In the group treated with furosemide, serum calcium and phosphorus and the calcium/phosphorus ratio did not differ from the congestive heart failure control group (Table 1). Nor did discriminant function analysis reveal any differences between the patients treated with furosemide and their controls in respect of calcium and phosphorus values (Fig. 2).

DISCUSSION

Hypocalcemia has been reported by several authors to occur during thiazide treatment. Lambert and Kuhlback (7) originally found decreased

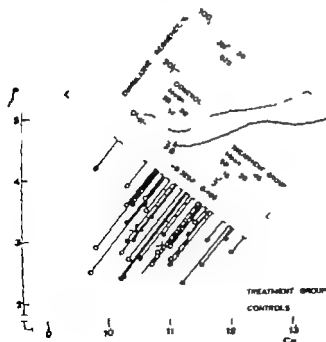


Fig. 1 Serum calcium and phosphorus concentrations (mg/100 ml) in hypertensive patients treated with (●) and (○) thiazides.

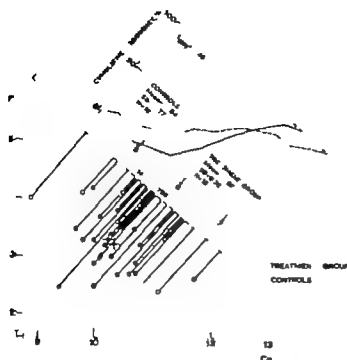


Fig 2 Serum calcium and phosphorus concentrations (mg/100 ml) in patients with congestive heart failure treated with (●) and without (○) furosemide.

calcium excretion during and after chlorothiazide and hydrochlorothiazide treatment. They also found a small but inconsistent increase in serum calcium values during the first few days of treatment. Lichtwitz et al. (8) reported hypocalcemia during thiazide treatment both in patients and in normal controls. Duarte and Bland (2) reported decreased excretion of calcium after oral ingestion of chlorothiazide, an effect also found when the drug was given by the intravenous route (1). Seitz and Jaworski (12) noticed a 59% decrease in the urinary output of calcium and a slight increase in serum calcium during the first few days of hydrochlorothiazide treatment, but the serum calcium concentration was not found to be significantly or consistently raised during chronic treatment. Recently Duarte et al. (3) have reported a case in which hypocalcemia and hypercalcaemia were induced by hydrochlorothiazide. In this case the rise in serum calcium was also transient and a normal level was found 2 weeks after the start of the treatment, although the decrease in urinary calcium was still sustained.

In the present study in which the patients had received thiazides for at least 3 months, serum calcium was slightly increased and significant differences between the serum calcium and phos-

phorus values of the patients treated with thiazides and the controls were disclosed by discriminant function analysis.

Furosemide, on the other hand, has been reported to induce hypercalciuria in normal volunteers, an effect suggested to be due to inhibition of tubular reabsorption of calcium (13). This effect on urinary excretion decreases when treatment is continued (15). We found that long-term furosemide treatment did not affect serum calcium and phosphorus values in congestive heart failure patients. However the possibility that furosemide could affect these values in hypertension patients cannot be excluded, because in the present study such groups could not be studied.

In chronic experiments Pickleman et al. (10) found that significant hypercalcaemia and hypophosphataemia developed in dogs receiving hydrochlorothiazide orally. The maximum calcium response was noted at 5 weeks and was followed by a gradual decline to normal levels. The parathyroid glands of these dogs were enlarged and showed areas of histological change suggestive of hyperactivity (11). This resembles the human case reported by Duarte et al. (3), in which hypercalcaemia eventually disappeared in spite of con-

tinued administration of hydrochlorothiazide. Hypercalcaemia due to parathyroid adenoma or hyperplasia was reported in eight patients receiving long term thiazide therapy (9), and it was suggested that thiazides may lead to parathyroid stimulation in human beings. But during thiazide treatment it has not been possible to demonstrate constant changes in phosphate excretion tubular resorption of phosphate, or phosphate or creatinine clearance, such as would be expected if parathyroids were involved (16). However when our data were analysed by the sensitive discriminant function analysis, the group treated with thiazide was found to differ from its control group with regard to the serum levels of both calcium and phosphorus. On the other hand, hydrochlorothiazide has been shown to increase serum calcium in patients on maintenance haemodialysis with negligible or absent renal function (6). Parathyroid extract failed to elicit a rise in serum calcium in most of the patients with uraemia who had a hypercalcaemic response to thiazides. These findings led Koppel et al. (6) to suggest that the extrarenal effect of thiazides on calcium metabolism may be due to potentiation of the action of parathyroid hormone on bone.

To clarify the mechanism of hypercalcaemia, either renal or extrarenal, direct measurement of parathyroid hormone in the blood during thiazide administration is needed.

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THE EFFECT OF BENDROFLUMETHIAZIDE ON TOTAL, ULTRAFILTRABLE AND IONIZED CALCIUM IN SERUM IN NORMOCALCAEMIC RENAL STONE FORMERS AND IN HYPERPARATHYROIDISM

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Abstract. The effect of orally administered bendroflumethiazide, 10 mg/24 h, on total, ultrafiltrable and ionized calcium in serum has been studied in 10 hyperparathyroid patients and 10 normocalcaemic renal stone formers to evaluate the usefulness of thiazide administration in the diagnosis of hyperparathyroidism. An increase in total calcium parallel to an increase in serum proteins was seen in both groups but without any change in either ultrafiltrable or ionized calcium. Thus the thiazide provocative test appears to be of no use in our hands. The disproportionate increase in total calcium, which has been noticed occasionally in hyperparathyroidism by some investigators, is discussed.

Oral administration of diuretic thiazidines and chlorothalidone decreases the renal excretion of calcium in man (2, 3, 6, 7, 13, 14, 24) and in experimental animals (9). Concomitantly a slight increase in serum calcium concentration has been noticed (2, 6, 13, 17, 24). In some hypercalcaemic patients, however, a disproportionately large increase in serum calcium has occurred (4, 15, 16, 18, 19, 23) and for this reason administration of thiazides has been recommended as a provocative test in cases of suspected hyperparathyroidism (1, 23). Since rather few patients with hyperparathyroidism have been studied, and both false negative and positive tests have appeared, the diagnostic value of this test remained uncertain. All previous studies have been based on total calcium determinations, rarely supplemented by determinations of serum ultrafiltrable calcium (2, 4), but determinations of serum ionized calcium have not been undertaken.

This investigation, which is a part of a comprehensive assessment of the effects of thiazide

diuretics on calcium metabolism (9, 10, 11, 12), presents the changes in serum total, ultrafiltrable and ionized calcium observed during thiazide administration to patients either suffering from hyperparathyroidism or being suspected of harbouring this disorder.

MATERIAL AND METHODS

Twenty patients (Table I) presenting with either well-planned renal insufficiency (patients 2 and 3), arterial hypertension (patient 5) or renal stone disease, and having either high normal or frankly elevated routine serum calcium, were selected for 10-day metabolic study. They were given standard diet containing approximately 40 mEq calcium, 1000 mg phosphate, 70-100 mEq sodium and 1 g protein/kg b.wt. day.

During the last 5 days bendroflumethiazide (Cenit 15) was administered in doses of 2.5 mg at 8 a.m. and noon and 5 mg at bedtime. Serial determinations of ionized calcium (Ca^{++}), ultrafiltrable calcium (UFCa), total calcium (TOCa), serum proteins, inorganic phosphate and creatinine in serum were carried out on days 3, 4, 5, 8, 9 and 10; 24-hour urine was collected throughout and analysed for calcium and creatinine. For diagnostic purposes the 24-hour tubular reabsorption of calcium (TRCa%) was calculated on the basis of TOCa, the clearance of creatinine and the urinary calcium excretion (20, 22).

According to the average concentration of Ca of the control period the patients were classified as hypercalcaemic (group 1, patients 1-10) and normocalcaemic (group 2, patients 11-20). In group 1 the diagnosis of hyperparathyroidism was based on 1) the presence of hypercalcaemia for which no other cause could be demonstrated, and 2) the finding of TRCa% values within the range of patients with hyperparathyroidism studied on similar diet (22). Since the determination of Ca is sensitive tool in the detection of cases of so called normocalcaemic hyperparathyroidism (31), this diagnosis was

Table 1. Control data of 10 hyperparathyroid patients (nos 1-10) and 10 normocalcaemic renal stone formers (nos 11-20)

Pat. no.	Sex	Age (y)	Serum concentration				Urinary excretion 24 h				Creatinine clearance (ml/min)	
			Ca (mEq/l)	UFC (mEq/l)	TOCa (mEq/l)	P (mg/100 ml)	TOMg (mEq/l)	UFMg (mEq/l)	Calcium (mEq)	Sodium (mEq)		Creatinine (mg)
1	F	54	3.43	3.66	3.99	2.7	1.84	1.53	16.9	62	1441	86
2	F	4	3.06	3.97	6.00	2.7	1.47	1.27	11.1	43	799	51
3	F	30	2.2	3.52	3.54	3.1	1.60	1.42	4.1	62	830	39
4	F	36	2.1	3.71	4.04	2.6	1.91	1.57	4.1	67	1490	179
5	F	36	2.96	3.3	3.35	2.6	1.30	1.01	5.4	7	1400	33
6	F	4	2.6	3.66	3.60	3.4	1.77	1.38	20.7	60	1215	97
7	F	64	2.4	3.57	3.75	3.0	1.43	1.14	22.9	81	1483	109
8	F	5	2.0	3.40	3.18	2.5	1.64	1.28	14.5	95	1477	99
9	F	6	2.6	3.45	3.15	3.0	1.70	1.37	14.1	105	1874	102
10	F	3	2.0	3.3	3.27	3.5	1.64	1.44	2.1	31	1100	31
11	F	6	2.6	3.26	3.10	3.1	1.79	1.41	6.1	105	1284	99
12	F	41	2.6	3.08	3.1	3.3	1.80	1.67	11.1	48	1677	72
13	F	51	2.6	3.0	3.11	4.3	1.76	1.31	5.8	131	1296	77
14	F	6	2.6	3.0	4.95	3.1	1.73	1.43	5.8	66	1284	82
15	F	5	2.0	3.0	3.10	2.5	2.29	1.18	17.3	64	1445	81
16	F	6	2.6	3.03	3.1	3.3	1.83	1.48	14.5	67	1365	117
17	F	6	2.6	3.0	4.1	3	1.73	1.36	15.1	117	1488	111
18	F	6	2.6	3.10	3.11	3.3	1.79	1.44	12.1	128	1881	123
19	F	5	2.6	3.10	3.07	4.3	1.79	1.57	9.1	126	1790	123
20	F	45	2.13	3.1	3.07	2.8	1.79	1.33	14.5	7	1495	114

from 1976 to 1978 for clinical purposes. Wilcoxon's test of paired differences and Wilcoxon test for two samples were used.

RESULTS

An increase in the renal excretion of calcium is observed after the pre-treatment level is reached in the first 24 h of thyroid administration in both normocalcaemic and hypercalcaemic patients. On the 2nd day of thyroid administration the renal and urinary calcium excretion is pre-treatment level. At the same time the effect on renal calcium excretion becomes maximal when urinary changes during thyroid administration (Fig. 1). The increase in total calcium excretion makes up about 50% of the pre-treatment level. However, the urinary calcium excretion is 50% higher on the 2nd day (Fig. 1).

The renal excretion of magnesium in the normocalcaemic and hypercalcaemic patients was equal prior to thyroid administration. 8 h after 24 h. In both groups the renal magnesium excretion increased during thyroid administration. During the 48 h of treatment the magnesium excretion returned to the pre-treatment level in the normocalcaemic group and in the hypercalcaemic group (Fig. 2). The increase in renal mag-

nesium excretion is accompanied by a decrease in TOmg and UFMg in serum (Fig. 4) secondary to a decrease in TRMg (Table III).

Although there is a slight decrease in the filtered load of calcium during thyroid administration, being significant in the normocalcaemic group (17.6 vs. 16.1 mEq), calculation of the whole handling of calcium shows that the reduction in urinary calcium excretion during thyroid administration is due to an increased TRMg (Table III).

Both in normocalcaemic and hypercalcaemic patients an increase in the urinary excretion of potassium and phosphate is seen during thyroid administration with a greater increase in normocalcaemic than in hypercalcaemic patients (Fig. 3). When the urinary excretion of calcium and phosphate is restored to the 24-hour control level the urinary excretion of potassium and phosphate is equal to the pre-treatment level in both groups during thyroid administration (Table 3).

DISCUSSION

Among our patients with the diagnosis of the hypercalcaemic phase of thyroid carcinoma

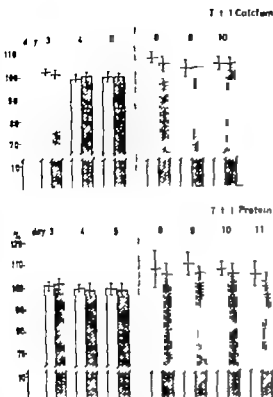


Fig. 1 Changes in serum proteins and TOCs in serum during bendroflumthiazide administration, 10 mg/24 h, in 10 normocalcaemic (open columns) and 10 hyperparathyroid patients (hatched columns) \pm S.D. 100% represents the average control level of days 3, 4 and 5.

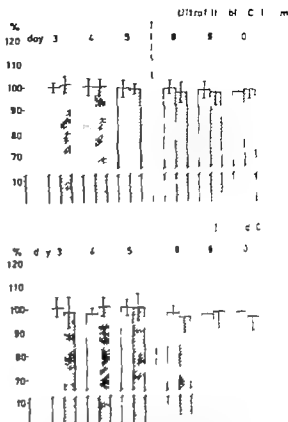


Fig. 2 Changes in UFCa and Ca in serum during bendroflumthiazide administration, 10 mg normocalcaemic (open columns) and 10 hyperparathyroid (hatched columns) \pm S.D. 100% represents the average control level of days 3, 4 and 5.

The values for each day of thiazide administration are compared with the average value of the three control days by Wilcoxon's test for pair differences and show a significant increase in TOCa and serum proteins ($p < 0.02$) on days 8, 9 and 10. During the control period serum TOCa averaged 5.62 mEq/l in group 1 against 5.05 mEq/l in group 2 (Table I). Therefore the identical change of +5% observed on day 9 (Fig. 1) represents of +0.28 and slightly different absolute changes of +0.28 and +0.25 mEq/l, respectively. There is a tendency to a decrease in UFCa and Ca during thiazide administration (Fig. 2), but a significant decrease appears in the normocalcaemic group only on the 5th day of thiazide administration ($p = 0.01$). The concentration of UFCa and Ca^{++} during thiazide administration did not exceed the highest value observed during the control period in any of the patients.

DISCUSSION

A slight increase in TOCa at the beginning of the treatment period, apparently due to increased serum proteins (2, 15), has been observed by several groups investigating the effect of diuretic thiazides on urine calcium excretion (2, 6, 13, 17, 24). In hypercalcaemia several reports of a greater increase in TOCa have been presented (4, 15, 16, 18, 19, 23). Although van der Veer *et al.* (23) did not find a significant increase in TOCa in 4 out of 9 hyperparathyroid patients, they suggested chlorthalidone administration as diagnostic test in hyperparathyroidism. Sode *et al.* (18) studied the effect of short-term thiazide administration in 7 patients with borderline and/or intermittent hypercalcaemia and found a significant increase in TOCa in all. In 5 of these patients parathyroid adenoma was found. Adams *et al.* (1)

have used chlorothiazide in combination with phosphate deprivation and found, in addition to 5 hypercalcaemic patients rendered hypercalcaemic by phosphate deprivation, 3 patients rendered hypercalcaemic by combining the phosphate deprivation with thiazide administration. Six of these patients were operated upon and a parathyroid adenoma was found in five. An increase exceeding 2 mg % in TOCa in serum has been seen in non-parathyroid hypercalcaemia as well (4 15 19).

The present study does not demonstrate any change in either UPCa or Ca of normocalcaemic or hyperparathyroid patients during short term thiazide administration. Only TOCa increases during thiazide administration, suggesting that the increase in TOCa is due to the increase in protein concentration. These results are in accordance with those of Brickman et al. (2) who found an identical increase in TOCa in hyperparathyroid normocalcaemic and hypoparathyroid patients during thiazide administration. Thus the thiazide provocative test appears to be of no value in the diagnosis of hyperparathyroidism.

ACKNOWLEDGEMENTS

This investigation was supported by grants from Jørgensen O. Beck's Fond, Christian X Fond, Nordisk Insulin-fond and P. Carl Petersen's Fond.

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THE EFFECT OF BENDROFLUMETHIAZIDE ON THE RENAL HANDLING OF CALCIUM MAGNESIUM AND PHOSPHATE IN NORMOCALCAEMIC RENAL STONE FORMERS AND IN HYPERPARATHYROIDISM

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Abstract. The effect of bendroflumethiazide (Cintyl®) on the renal excretion of calcium, magnesium and phosphate has been investigated in 10 normocalcaemic renal stone formers and 10 hyperparathyroid patients to evaluate the effect of diuretic thiazides on calcium metabolism. A significant increase in the tubular reabsorption of calcium and significant decrease in the tubular reabsorption of magnesium was observed in both groups. A reduction of 52% in renal calcium excretion was found in both groups, being independent of supposed differences in parathyroid hormone secretion but significantly related to the pretreatment urinary calcium excretion. The renal excretion of magnesium and phosphate increased during thiazide administration. Different theories concerning the mechanism through which diuretic thiazides act on calcium metabolism are discussed. Based on the present results combined with previous investigations, both direct effect on tubular calcium reabsorption and an effect on bone metabolism are postulated.

Oral administration of thiazide diuretics produces a fall in urinary calcium excretion in man (3, 12, 22, 28, 29, 41) and experimental animals (16). This action, which is believed to result from an enhanced tubular reabsorption of calcium (22) is independent of the parathyroid glands in rats (16) but is said not to be so in man (3, 30). Besides this renal effect of thiazides, a hypercalcaemic effect observed under certain conditions (21, 29, 36, 38) has led to the suggestion of a potentiating effect of this drug on the bone action of parathyroid hormone (21, 29). The known renal effects of parathyroid hormone are to increase the tubular reabsorption of calcium (2, 20) and magnesium (24, 26) and to inhibit the tubular reabsorption of phosphate (13, 14).

The aim of the present investigation has been to study the renal handling of calcium, magnesium

and phosphate during thiazide administration in normocalcaemic and hypercalcaemic patients.

MATERIAL AND METHODS

Ten normocalcaemic renal stone formers and 10 hyperparathyroid patients were investigated for 10 days (Table I). Bendroflumethiazide (Cintyl®) was given for the last 4 days, 2.5 mg at 8 a.m. and noon, 5 mg at bedtime. The normocalcaemic patients were all suspected of having hyperparathyroidism because of routine serum calcium determinations on the upper part of the normal range, but turned out to have normal total (TOCa), ultrafiltrable (UFCa) and ionized (Ca^{++}) calcium concentrations (deter-mined in the research laboratory). In the hyperparathyroid patients other causes of hypercalcaemia were excluded by 1) thorough medical examination and 2) tubular reabsorption of calcium (TRCa%) within the range of patients with hyperparathyroidism (starch on toadlet diet (37) consisting of 40 mEq calcium, 1 000 mg phosphate, 70-100 mEq sodium and 1 g protein/kg b.wt./day).

The serum concentration of sodium, potassium, chloride, total CO_2 , proteins, creatinine and phosphate, and the urinary content of sodium, potassium, creatinine, calcium, magnesium and phosphate, were determined daily. On days 3, 4 and 5 and 8, 9 and 10 the concentration of TOCa, UFCa, Ca^{++} , total magnesium (TOMg) and ultrafiltrable magnesium (UFMg) in serum was measured. The tubular reabsorption of calcium, magnesium and phosphate expressed as percentage of the filtered load of calcium, magnesium and phosphate (TRCa%, TRMg%, TRP%) were calculated from the serum concentration of UFCa, UFMg and phosphate and the urinary excretion of creatinine, calcium, magnesium and phosphate on days 3, 4, 5, 8, 9 and 10.

Sodium and potassium were determined by flame photometry (Technicon AutoAnalyzer IV), total CO_2 , chloride and creatinine by Technicon AutoAnalyzer N-methodology, phosphate by the method of Goldenberg and Fernandez (9). Protein was determined by UV spectrophotometry (15). TOCa, UFCa, Ca, TOMg and UFMg by atomic absorption, pressure ultrafiltration and cal-

Table I Control data of 10 hyperparathyroid patients (nos 1-10) and 10 normocalcaemic renal stone formers (nos 11-20)

Pat. no.	Sex	Age (y.)	Serum concentration				Urinary excretion 24 h					Creatinine clearance (ml/min)
			Ca ⁺⁺ (mEq/l)	UPCa (mEq/l)	TOCa (mEq/l)	P (mg/100 ml)	TOMg (mEq/l)	UFMg (mEq/l)	Calcium (mEq)	Sodium (mEq)	Creatinine (mg)	
1	♂	54	3.43	3.66	5.59	2.7	1.86	1.53	16.9	62	1.441	86
2	♀	74	3.06	3.97	6.00	2.7	1.47	1.27	11.2	43	799	57
3	♂	80	2.73	3.57	5.54	3.1	1.60	1.42	4.2	82	850	29
4	♂	49	2.71	3.71	6.04	2.6	1.91	1.57	24.1	67	1.490	110
5	♀	39	2.69	3.78	5.88	2.6	1.30	1.01	5.4	72	1.400	23
6	♀	24	2.69	3.69	5.60	3.4	1.72	1.38	20.7	60	1.235	97
7	♀	69	2.64	3.57	5.75	3.9	1.45	1.14	22.9	82	1.483	109
8	♂	52	2.60	3.40	5.18	2.5	1.64	1.28	14.5	95	1.477	109
9	♂	52	2.56	3.45	5.35	3.0	1.70	1.37	14.2	105	1.834	102
10	♂	58	2.50	3.23	5.27	3.5	1.64	1.44	4.2	81	1.100	51
11	♂	56	2.46	3.29	5.10	3.1	1.79	1.41	6.2	105	1.284	93
12	♂	41	2.42	3.08	5.17	3.3	1.80	1.47	11.1	48	1.677	72
13	♀	51	2.56	3.02	5.11	4.3	1.76	1.31	5.8	131	1.296	77
14	♂	56	2.36	3.06	4.95	3.1	1.73	1.43	5.8	66	1.284	92
15	♂	37	2.50	3.06	5.10	3.5	1.59	1.18	17.3	89	1.445	81
16	♂	54	2.27	3.05	5.16	3.3	1.83	1.48	14.5	67	1.865	147
17	♂	54	2.27	2.98	4.77	3	1.75	1.36	15.2	117	1.488	118
18	♂	29	2.16	3.18	5.11	3.3	1.78	1.39	12.1	128	1.861	121
19	♂	22	2.15	3.18	5.00	4.1	1.73	1.37	9.1	126	1.760	113
20	♂	45	2.13	3.12	5.01	2.8	1.76	1.33	14.5	77	1.493	114

cann electrode (19). For statistical purposes Wilcoxon's test of paired differences and Wilcoxon's test for two samples were used.

RESULTS

An increase in the renal excretion of sodium of about 300% of the pretreatment level occurring on the first day of thiazide administration is seen in both normocalcaemic and hypercalcaemic patients. On the 3rd day of thiazide administration renal sodium excretion returns to the pre-level. At the same time the effect on calcium excretion becomes maximal without further change during thiazide administration (Fig. 1). The reduction in renal calcium excretion makes up about 52% of the pretreatment level irrespective of the urinary calcium excretion before thiazides ($p < 0.01$) (Fig. 2).

The renal excretion of magnesium of the normocalcaemic and hypercalcaemic patients was equal prior to thiazide administration, 8.5 mEq/24 h. In both groups the renal magnesium excretion increases during thiazide administration but during continuing treatment the magnesium excretion returns to the pretreatment level on the 5th thiazide day in the normocalcaemic group ($p > 0.10$) (Fig. 3). The increase in renal mag-

nesium excretion is accompanied by a decrease in TOMg and UFMg in serum (Fig. 4) secondary to a decrease in TRMg% (Table II).

Although there is a slight decrease in the filtered load of calcium during thiazide administration, being significant in the normocalcaemic group ($0.05 < p < 0.02$), calculation of the tubular handling of calcium shows that the reduction in urinary calcium excretion during thiazide administration is due to an increased TRCa% (Table III).

Both in normocalcaemic and hypercalcaemic patients an increase in the urinary excretion of potassium and phosphate is seen during thiazide administration, with a greater increase in normocalcaemic than in hypercalcaemic patients (Fig. 5). When the urinary excretion of potassium and phosphate is related to the 24-hour creatinine excretion, the urinary excretion of potassium and phosphate is equal in the two groups ($p > 0.10$). A hypochloreaemic, hypokalaemic alkalosis develops in both groups during thiazide administration (Table IV).

DISCUSSION

Lamberg and Kuhlþák (22) the discoverers of the hypocalcaemic effect of thiazides, considered an

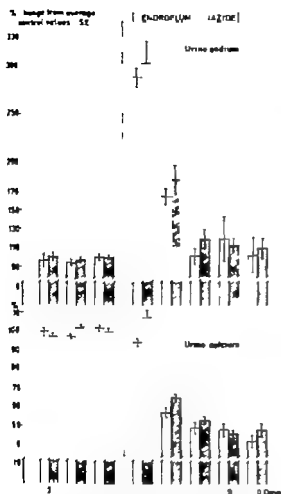


Fig. 1 Effect of bendroflumethiazide (10 mg/day) on urinary calcium and sodium excretion in 10 normocalcaemic renal stone formers (□) and 10 hyperparathyroid patients (■). 100% represents the average control level of days 3, 4 and 5.

increased TRCa% to be the most likely explanation for their observation. This hypothesis is now confirmed in the present study. The mechanism(s) through which the reabsorption is enhanced remains a matter of dispute and several theories have been advanced.

Extracellular fluid volume contraction enhances TRCa% and has therefore been considered an important factor in the hypocalcaemia of thiazide administration (3, 34). This hypothesis was based on the fact that a high salt intake abolished the hypocalcaemic response to thiazides in normocalcaemic individuals. The same data may lead to a quite different interpretation, namely that the

Table II. Fractional TRMg% and TRP% in 10 hyperparathyroid patients and 10 normocalcaemic renal stone formers during bendroflumethiazide administration (10 mg/day) compared with the average value of 3 control days by Wilcoxon test of paired differences

	Pre-treatment average	Day of bendroflumethiazide administration		
		3	4	5
Hyperparathyroid patients				
TRMg%, \bar{x}	93.7	91.6	89.0	90.5
S.D.	3.6	2.7	4.1	3.1
<i>P</i>		<0.01	<0.01	<0.01
Normocalcaemic patients				
TRMg%, \bar{x}	95.7	94.3	92.0	94.9
S.D.	1.0	1.4	2.7	1.7
<i>P</i>		<0.01	<0.01	0.05 < <i>P</i> < 0.10
Hyperparathyroid patients				
TRP%, \bar{x}	68.6	69.1	66.6	64.4
S.D.	13.7	11.9	10.1	13.4
<i>P</i>		>0.10	>0.10	0.05 < <i>P</i> < 0.10
Normocalcaemic patients				
TRP%, \bar{x}	61.3	78.1	61.1	78.0
S.D.	13.4	5.6	7.8	7.2
<i>P</i>		0.05 < <i>P</i> < 0.10	=0.05	>0.10

excessive renal output of sodium has masked the intensifying effect of thiazides on TRCa%. This interpretation, being based on the well established interrelations between the renal handling of calcium and sodium (39), may also explain why the urinary calcium excretion decreases stepwise in order to reach its minimum when the urinary sodium excretion returns to normal after about 3 days of thiazide administration (Fig. 1).

The possible role of hypomagnesaemia also deserves comment. This deviation, which is caused by a decrease in TRMg% as shown in the present paper could act through a stimulation of parathyroid hormone secretion (4, 32). However conflicting and very convincing observations have also been reported (33). The observations on the end-organ responsiveness to parathyroid hormone during hypomagnesaemia also remain controversial (7, 11, 25, 33).

At first glance the role of parathyroid hormone secretion also seems debatable. On the one hand, one of us has reported an obvious hypocalcaemic reaction to thiazides in rats deprived of parathyroid tissue (16) while on the other hand Brickman et al. (3) and Parfitt (30) were unable to demonstrate any decrease in urinary calcium ex

Renal Co-excretion, mEq / 24 hrs,
during bendroflumethiazide
administration

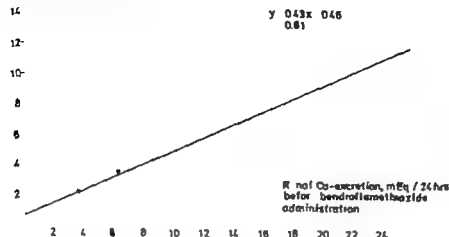


Fig. 2. Relationship between urinary calcium excretion before and during bendroflumethiazide administration, 10 mg/day in 10 normocalcaemic renal stone formers and 10 hyperparathyroid patients.

cretion during administration of thiazides to hypoparathyroid patients treated with vitamin D. A closer inspection of the data of Parfitt (30) which permit calculation of the tubular reabsorption of calcium, reveals, however, that TRCa% remains unchanged. Since a decrease in TRCa% is expected when serum calcium increases as in this situation (20), TRCa% increases relatively despite the absence of parathyroid hormone. Thus the animal and human studies do not appear to be contradictory.

A hypothesis of a potentiating action of thiazides on parathyroid hormone has also been advanced (21-29). This theory which is based on

the disproportionate increase in serum calcium in some hyperparathyroid patients treated with thiazides (1, 31, 36, 38) neglects the fact that a similar reaction has been noticed in patients with non-parathyroid hypercalcaemia supposedly having a depressed parathyroid hormone secretion (29, 31, 36). Furthermore, this hypothesis is incompatible with the experiments of Koppell et al. (21) in anephric patients and of Jørgensen (17) in rats. The observations in the present study showing dependence of the hypocalcaemic response on the pretreatment urinary calcium excretion, regardless of the group to which the patient belongs (Fig. 2), also seem hard to reconcile with a potentiating action of thiazides on parathyroid hormone.

In our opinion the enhancing action of thiazides on TRCa% appears to be a direct one although not the only action of thiazides on calcium metabolism. The study of Koppell et al. (21) strongly suggests a calcium-mobilizing action of thiazides upon the skeleton. Despite this action, and despite the renal retention of calcium, serum-ionized calcium remains unchanged in normocalcaemic subjects (19). The only two ways in which this can be accomplished is by stimulation of calcitonin secretion and suppression of parathyroid secretion, both leading to a decrease in bone resorption and possibly to a decrease in the intestinal absorption of calcium—or by a direct inhibitory effect of thiazides on the latter. The evidence for an action of thiazides on calcium absorption, direct or indirect, is most controversial

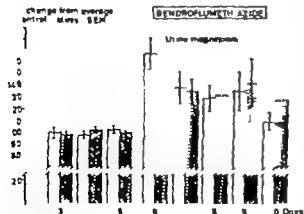


Fig. 3. Urine magnesium in 10 normocalcaemic renal stone formers (□) and 10 hyperparathyroid patients (■) during bendroflumethiazide administration, 10 mg/day. 100% represents the average control level of days 3, 4 and 5.

Table III. Renal handling of calcium before and during bendroflumethiazide (TZ) administration, 10 mg/day in 10 hyperparathyroid patients (nos. 1-10) and 10 normocalcaemic renal stone formers (nos. 11-20)

Pat. no.	Serum UFCa (mEq/l)		Creatinine clearance (ml/min)		Filtered Ca (mEq/24 h)		Urinary calcium (mEq/24 h)		Fractional TRCa	
	Control	TZ	Control	TZ	Control	TZ	Control	TZ	Control	TZ
1	3.66	3.72	86	77	453.1	412.4	16.8	9.6	96.2	97.5
2	3.98	3.94	58	56	332.3	317.6	11.8	5.0	96.6	98.4
3	3.52	3.60	29	32	146.8	163.8	3.9	3.1	98.6	98.9
4	3.73	3.82	110	132	590.8	726.0	24.9	12.4	95.9	97.9
5	3.78	3.78	33	33	179.5	179.5	6.9	1.8	96.9	97.7
6	3.69	3.50	97	99	515.3	498.9	16.9	8.6	96.0	98.0
7	3.57	3.58	110	86	565.4	443.2	22.6	8.5	95.9	97.6
8	3.40	3.09	110	121	538.5	538.2	14.5	8.5	97.2	98.4
9	3.45	3.21	103	100	511.6	482.2	14.3	9.4	97.0	98.7
10	3.23	3.10	51	38	237.1	169.6	3.8	2.1	98.3	98.5
<i>P</i>	>0.10		>0.10		>0.10		<0.01		<0.01	
11	3.29	3.13	94	82	445.2	369.5	9.9	4.2	98.6	98.9
12	3.08	3.13	72	73	319.2	528.8	10.6	6.7	97.3	97.6
13	3.02	3.04	77	74	334.8	323.8	5.3	1.8	98.2	99.4
14	3.06	3.11	93	78	409.6	349.2	6.3	3.4	98.6	98.7
15	3.07	3.02	91	94	402.1	408.6	16.6	4.7	95.7	98.4
16	3.05	3.09	137	119	601.6	529.4	14.7	2.3	97.7	99.3
17	2.99	2.95	118	102	508.3	433.2	15.1	8.3	96.9	97.7
18	3.18	3.01	122	110	558.5	476.7	12.0	8.3	97.8	98.1
19	3.18	2.97	119	127	517.3	541.2	8.6	2.1	96.2	99.4
20	3.12	3.04	112	97	503.1	424.5	15.2	8.8	97.0	98.3
<i>P</i>	>0.10		=0.10		0.02 < <i>P</i> < 0.05		<0.01		<0.01	

(6, 10, 12, 23, 28) while the evidence for a decreased bone excretion secondary to hormonal counterregulation includes 1) a reduction in serum parathyroid hormone concentration and urinary hydroxyproline excretion during thiazide treatment of idiopathic hypercalcaemia (5-40), 2) a decreased excretion of hydroxyproline in rats during thiazide administration (18) and 3) an increased concentration of ^{45}Ca in bones of rats

following such treatment (18). The importance of the hormonal counterregulation for the maintenance of normocalcaemia despite thiazide administration is stressed by the development of hypercalcaemia in states of hormonal dysregulation. This happens regularly in hypoparathyroidism treated by vitamin D (3, 30) and occasionally in a variety of hypercalcaemic disorders (29, 31, 36, 38). In the former condition not only have the

Table IV. Average serum concentration of potassium, chloride and total CO_2 in 10 hyperparathyroid patients (h) and 10 normocalcaemic renal stone formers (n) during 5 control days and 5 days of bendroflumethiazide administration, 10 mg/day (S.D. within parentheses)

	Control days					Bendroflumethiazide days				
	1	2	3	4	5	6	7	8	9	10
Potassium (mEq/l)										
h	4.27 (0.21)	4.12 (0.39)	4.15 (0.40)	4.15 (0.44)	4.25 (0.36)	3.92 (0.40)	4.00 (0.39)	3.99 (0.32)	3.37 (0.49)	3.37 (0.39)
n	4.22 (0.34)	4.28 (0.31)	4.16 (0.31)	4.22 (0.33)	4.37 (0.47)	4.00 (0.37)	3.77 (0.42)	3.56 (0.30)	3.50 (0.78)	3.29 (0.55)
Total CO_2 (mmol/l)										
h	24.6 (2.4)	27.6 (2.0)	26.7 (2.1)	27.7 (1.9)	28.1 (2.4)	28.0 (2.4)	29.5 (5.8)	29.5 (3.4)	31.3 (3.3)	32.9 (2.5)
n	26.1 (2.9)	26.8 (2.8)	26.5 (1.9)	27.5 (1.8)	26.1 (1.8)	27.6 (1.5)	29.9 (2.8)	29.5 (2.4)	30.4 (2.8)	31.9 (4.3)
Chloride (mEq/l)										
h	109.5 (3.9)	106.9 (4.2)	106.0 (3.4)	108.1 (1.8)	107.6 (3.6)	94.9 (2.5)	97.9 (3.6)	98.2 (3.3)	98.2 (4.1)	97.1 (3.4)
n	109.8 (2.3)	109.9 (2.5)	110.1 (3.7)	108.7 (3.4)	105.5 (6.7)	99.4 (3.6)	101.2 (5.2)	100.5 (3.2)	99.7 (3.1)	98.5 (3.0)

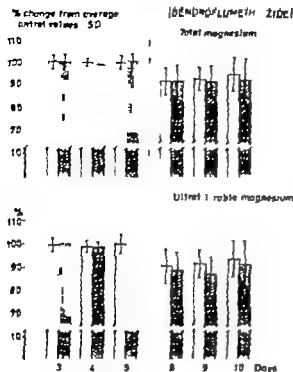


Fig. 4. Changes in the concentration of TOMg and UFMg in serum during bendroflumethizide administration, 10 mg/day in 10 normocalcaemic renal stone formers (□) and 10 hyperparathyroid patients (■). 100% represents the average control level of days 3, 4 and 5.

parathyroids been removed, but also the thyroid in many cases (30) and, therefore, the calcium-mobilizing effect of thiazides on bone works unopposed by compensatory changes in hormone secretion. Serum calcium, and thereby the filtered load of calcium, increases, ultimately tending to mask the effect of thiazides on the tubular reabsorption of calcium. In hypercalcaemic states a situation is quite similar so far as the parathyroid hormone is concerned, since the secretion of this hormone remains either inappropriately high or definitely low. Consequently accentuation of hypercalcaemia should follow thiazide administration, but this is rarely the case at least in moderate hypercalcaemia (3, 19). An adequate reserve capacity for calcitonin secretion could account for this finding and, conversely, exhaustion of this capacity (8, 35) offers a likely explanation of the rare instances of accentuated hypercalcaemia.

Brickman et al. (3) found a greater increase in urinary phosphate excretion during thiazide administration in normal subjects compared with a

hyperparathyroid group. The same phenomenon is seen in the present experiments without any definite change in TRP% (Table II), along with an increased potassium excretion in the normocalcaemic group. Most of the body potassium and the readily available phosphate is located intracellularly and a close relationship exists between total exchangeable potassium, lean body mass and urinary creatinine (27). When the urinary excretion of potassium and phosphate is expressed per unit of creatinine excretion, the difference between the two groups vanishes completely. Therefore we believe that the observed differences with respect to phosphate and potassium excretion merely reflect differences in lean body mass.

The mechanism of action of diuretic thiazides on calcium metabolism appears to be complex, involving both a direct effect on kidneys and bones in addition to secondary changes in the hormonal control of calcium homeostasis. Further

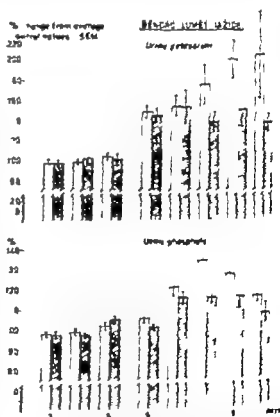


Fig. 5. Urinary excretion of potassium and phosphate during bendroflumethizide administration, 10 mg/day, in 10 normocalcaemic renal stone formers (□) and 10 hyperparathyroid patients (■). 100% represents the average control level of days 3, 4 and 5.

studies of the intestinal calcium absorption and determinations of the parathyroid hormone and calcitonin in serum during thiazide administration are necessary to further elucidate the mechanisms involved.

ACKNOWLEDGEMENTS

This investigation was supported by grants from Ingemar O. Bock, Pönd, Christina X. Pönd, Nordisk Insulin-fund and P. Carl Petersen's Fond.

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Table I. Some general data and thyroid function tests in patients euthyroid after treatment for hyperthyroidism

Pat. no.	Sex	RAI dose (mCi)	Time since treatment (yr)	FBI (μ g/100 ml)	T U (%)	FTI	T (μ g/100 ml)	Cholesterol (mg/100 ml)	TgA	TSH (μ U/ml)	TRH test	
											Basal TSH (μ U/ml)	Δ TSH ^b (μ U/ml)
Group 1 (elevated serum TSH)												
1	♀	4	4	7.8	1.00	78	9.8	240	5	7.6	8.7	61.3
2	♀	18	4	5.2	1.00	52	5.4	260	25 000	8.1	8.2	44.4
3	♂	4	2	5.2	0.88	47	6.3	200	—	8.1	49.0 ^c	451.0
4	♀	5	10	7.5	0.92	69	10.2	265	—	7.2	8.1	15.1
5	♀	32	10	6.7	0.94	63	8.4	298	—	7.2	8.2	9.5
6	♀	13	5	6.5	0.71	46	7.3	425	2 500	10.7	5.4	64.6
7	♀	57	4	7.2	0.72	52	7.4	345	2 500	7.2	4.5	20.9
8	♀	10	4	5.9	0.94	55	6.5	298	—	8.3	14.8	297.0
9	♀	5	5	6.0	0.86	52		220	280	16.0		
Group 2 (normal serum TSH and TgA > 25)												
10	♀	9	4	7.0	0.91	64		302	25	3.7	5.5	17.2
11	♂	21	4	6.9	0.97	67		265	25	1.7	4.0	6.0
12	♀	15	3	6.3	0.96	60		315	290	3.0	6.4	29.6
13	♀	13	1	8.5	0.91	79	8.7	390	2 900	4.4	6.1	16.6
14	♀	10	1	5.4	0.91	59		225	290	5.5	7.3	23.2
15	♂	11	3	4.8	1.10	53	5.1	275	25	2.7	2.2	3.2
16	♀	8	1	7.7	0.92	71		185	290	3.3	3.8	8.0
17	♂	27	1	7.7	1.10	85		225	25	3.7	3.7	0.0
Group 3 (normal serum TSH without detectable TgA)												
18	♀	8	1	7.6				340	—	5.2	4.2	14.8
19	♀	3	4	6.0	1.01	61		265	—	3.7	3.0	6.4
20	♀	20	5	5.8	0.78	45		180	—	3.7	4.2	34.8
21	♀	18	10	9.6	0.86	83	15.2	265	—	6.8	9.8	12.5
22	♀	10	5	7.9	0.84	70		280	—	3.0	2.5	8.8
23	♀	8	1	8.7	0.95	83	7.4	245	—	3.7	1.7	4.4
24	♀	10	10		0.93		7.4	285	—	3.7	2.5	5.2
25	♂	14	4	6.0	1.02	81		230	—	3.0	2.0	3.4
26	♂	15	1	5.2	0.99	51		225	—	3.0	2.0	2.8
27	♀	18	3	5.1	0.90	50		190	—	1.7	1.7	0.4
28	♂	20	1	7.6	0.95	80		205	—	1.7	1.4	0.1

Inverse titre. ^b Increment in serum TSH. Hypothyroid.

were within normal limits. In many instances, however, measurement of total thyroxine (T₄) was considered, since the history often revealed iodine contamination and an artificially elevated FBI.

Serum TSH was measured in all 72 patients. In 27 of them the TRH test was performed about 3 months after the initial study. In but one of the 9 patients with an elevated serum TSH at the initial examination (group 1), in all 8 patients with a normal serum TSH and a positive thyroglobulin antibody (TgA) titre >1/25 (group 2), and in 11 randomly selected patients with a normal serum TSH but without TgA (group 3). The sex, age, time elapsed since radioiodine treatment and total dose of radioiodine received by the patients in the three groups are shown in Table I.

Methods

Serum FBI was measured by the AutoAnalyzer Technicon N-56 method (normal range 4.0–8.0 μ g/100 ml) and T₄U was determined according to the method of Hansen

(29), as modified by Nossin (41) (normal range 80–120%). The free thyroxine index (FTI) was calculated as described by Clark and Horn (11) (normal range 40–80). T was determined by the competitive protein-binding technique of Murphy (39) as modified by Lleverstad et al. (37) (normal range 3.9–10.7 μ g/100 ml). T was measured in all subjects with FBI values below 5.0 and above 8.0 μ g/100 ml or when the serum TSH was above 6.9 μ U/ml. Serum total triiodothyronine (T₃) was determined by the method of Sterling et al. (58) as modified by Skovsted and Christensen (personal communication) (normal range 67–139 ng/100 ml). Determination of serum TSH was carried out by the double antibody radioimmunoassay as described earlier (22) (human TSH and TSH antiserum were donated by the Pituitary Agency of the National Institutes of Health, Bethesda, USA, and human TSH standard by the National Institute for Medical Research, London, England). Since the distribution of normal values was skewed, a logarithmic transformation was used for calculating the mean

and standard deviation (S.D.). The normal range is thus (\pm S.D.) 1.6–6.9 μ U/ml, with a mean of 3.4 μ U/ml.

The TRH stimulation test was performed as follows (42): 200 μ g synthetic TRH (Hoffman-La Roche Basel, Switzerland) was given intravenously as rapid injection. Serum TSH was determined before and 20 and 40 min after the injection. The normal values in 25 euthyroid TRH-stimulated control subjects were 2.8 ± 1.2 , 15.4 ± 8.0 and 11.0 ± 6.7 μ U/ml, respectively. A maximal rise at 20 min of more than 30.0 μ U/ml was regarded as abnormally high and response of less than 3.0 μ U/ml as abnormally low.

The presence of TgA in the circulation was determined by the brominated red cell technique (45). Long-acting thyroid stimulator (LATS) was determined by modification of the method of McKerrill (32, 33).

Serum cholesterol was measured by the Technicon Auto-Analyzer N-77 method.

RESULTS

The basal serum TSH value was elevated in 9 (13%) of the 72 euthyroid patients previously treated with radioiodine for hyperthyroidism. The elevated values ranged from 7.2 to 16.0 μ U/ml (Table I).

Five (56%) of the 9 patients with an elevated serum TSH value had circulating TgA, whereas only 12 (19%) of the remaining 63 patients with a normal serum TSH had TgA. Those with an elevated serum TSH also had the highest TgA titres (Table I).

Of the 12 patients originally treated for TDG 5 (42%) had an elevated TSH level, whereas only 4 (7%) of the 60 patients treated for TNG had an elevated serum TSH value (Table I).

There was no correlation between the serum TSH level and the time elapsed since treatment, although those with an elevated basal serum TSH level had been treated earlier than the rest. Neither was there a correlation between the serum TSH values and the total dose of radioiodine given for the treatment of hyperthyroidism. However, the 5 patients with an elevated serum TSH level and diffuse goitre had received much smaller total doses of radioiodine than those with an elevated serum TSH and nodular goitre (Table I).

The response to TRH was exaggerated in 5 of the 8 patients who had an elevated serum TSH level at the first examination (group 1), although in one case (no. 7) the basal serum TSH level was normal on the occasion of the TRH test. Another of these patients (no. 3) had meanwhile gone into clinically evident hypothyroidism

with low thyroid function tests (PBI 3.9 μ g/100 ml) and the basal serum TSH had risen from 8.1 to 49.0 μ U/ml. In 3 patients of this group the response to TRH was normal, in 2 of them the basal serum TSH was slightly elevated when first studied but normal at the time of the TRH test, and in one it was still slightly above the normal upper limit (Table I).

In 6 of the 8 patients with a normal serum TSH when first examined and with positive TgA titres ($>1/5$) (group 2) the response to TRH was normal. In one of them (no. 14), however the basal serum TSH had risen slightly above the normal limit. The response to TRH was absent in 2 patients in this group (nos. 16 and 17).

The response to TRH was normal in 8 of the 11 patients with a normal serum TSH (at the first examination) without circulating TgA (group 3), although in one of them (no. 21) the basal serum TSH had risen above the normal upper limit. The response was below normal in 3 patients in this group (nos. 26, 27 and 28) and in patient 28 even the basal serum TSH level had declined to subnormal levels.

The patients with absent response to TRH were studied in greater detail. Patient 17 had originally been treated for toxic multinodular goitre. He was taking sedatives which may have masked some of the symptoms and signs of hyperthyroidism and even possibly interfered with the TRH test. Originally patients 26 and 27 had had a solitary hyperactive autonomous adenoma. At the time of the study they were not taking any drugs regularly. Patients 16 and 28 had been treated for toxic multinodular goitre. They were not taking any drugs at the time of the study. LATS activity could not be found in the serum of any of these patients; the serum T_4 concentration was normal in 4 of them (nos. 17, 26, 27, 28), 107, 131, 88 and 101 ng/100 ml, respectively, and in patient 16 it was not measured.

DISCUSSION

Treatment of hyperthyroidism with radioactive iodine was introduced into Finland in 1954. Owing to the predominance of TNG in Finland, surgery is usually the treatment of choice, but radioactive iodine proved useful in certain groups of patients (31, 34, 53).

Hypothyroidism is the most important sequel

of radioactive iodine therapy but the total incidence varies greatly in different series. The risk of hypothyroidism is greatest after the first year of radiiodine treatment, but the cumulative incidence seems to increase continuously at an average rate of 2% per year (3, 6, 9, 10, 12, 14, 15, 18, 19, 23, 27, 40, 46, 53). To minimize the risk of hypothyroidism some have recommended smaller doses of radiiodine (24, 27, 49) and the treatment has been fractionated (19, 30, 44). TNG has proved more resistant to radiiodine treatment than TDG (10, 16, 19, 28, 34, 35, 43, 53).

The incidence of hypothyroidism after treatment of hyperthyroidism with radiiodine at Maria Hospital, Helsinki, (where the present study was performed) in 1959-69 was 25% in TDG and 12% in TNG after one year's follow-up and 35% and 21% respectively after 8 years follow-up (53).

The occurrence of subclinical hypothyroidism after radiiodine therapy has recently been reported. Slingerland et al. (47, 48) found that about half their euthyroid radiiodine-treated patients had elevated serum TSH values. During a population study in Finland it was noted that the prevalence of subclinical hypothyroidism in such patients was about 25% (21). In the present study only 13% of the patients had elevated TSH values. It thus seems that the prevalence of subclinical hypothyroidism varies considerably from one study to another. Follow-up studies should be done to explore whether these patients run risk of developing frank hypothyroidism. In

the present study one patient with subclinical hypothyroidism clearly became hypothyroid 3 months after the first examination.

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DIAGNOSTIC VALUE OF SERUM THYROXINE AND FREE THYROXINE INDEX

K. Liewendahl, R. Ruutu and B. A. Lamberg

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Abstract. A modified method for determination of serum total thyroxine (T_4) using competitive protein-binding reported. This method proved particularly valuable when analyzing large number of serum samples. A clinical evaluation of T_4 was performed in various thyroid disorders. T_4 was found to be superior to serum protein-bound iodine (PBI) in hyperthyroidism, mainly because of frequently encountered spuriously increased PBI values. In hypothyroidism PBI proved to be more accurate than T_4 and there were many patients with low-normal T_4 values who benefited by administration of thyroid hormone. Three different free thyroxine indices were studied. One as similar to the original free thyroxine index, here abbreviated FT_4 , defined as the product of PBI and triiodothyronine uptake by Sephadex (T_3U). Another free thyroxine index (FT_4I) was calculated as the product of T_4 and thyroxine uptake by Sephadex (T_4U). The product of T_4 and T_3U was the third free thyroxine index (FT_4I) studied. Of these indices FT_4 had the lowest diagnostic accuracy in both hyper and hypothyroidism. FT_4I and FT_4I were of approximately equal value in hyper and hypothyroidism. The clinical usefulness of both FT_4I and FT_4I was superior to that of T_4 in all conditions studied. It is recommended that both of these free thyroxine indices are determined when mild hyper or hypothyroidism is suspected.

The determination of serum total thyroxine (T_4) utilizing the specific binding properties of thyroxine-binding globulin (TBG) was first described by Ekins (8). The first method for clinical use was reported by Murphy and Pattee (27) and in a simplified modification by Murphy (26). A number of modified methods based on the same principle of competitive protein-binding analysis have since been published (4, 13, 29, 30, 36, 40). The determination of serum protein-bound iodine (PBI) has to a large extent been replaced by the measurement of T_4 because, unlike PBI it is not affected by the ingestion of iodinated com-

pounds. The diagnostic usefulness of T_4 has been documented in several investigations (2, 5, 12, 19, 28, 35).

It is generally accepted that the metabolic action of thyroxine is not determined by the total circulating thyroxine concentration but by the non-protein-bound free part. Robbins and Rall (32) estimated the free thyroxine concentration from electrophoretic data, and the first attempt to measure free thyroxine by a dialysis method was reported by Korsgaard Christensen (16). The free thyroxine concentration can be calculated after the determination of total thyroxine and the free thyroxine fraction. The correct measurement of the free thyroxine fraction calls for an equilibrium dialysis method (9, 14, 20, 21, 31, 39). Dialysis methods are rather laborious and, instead of determining the free thyroxine concentration a free thyroxine index is mostly used for routine purposes.

The free thyroxine index was devised from the observation that variations in the binding capacity of the thyroxine-binding proteins (TBP) often caused alterations in the concentration of circulating thyroid hormone (measured as PBI) and the number of free thyroxine-binding sites of TBP (estimated by the triiodothyronine resin uptake test) but that the product of PBI and triiodothyronine resin uptake remained constant. This original free thyroxine index of Clark and Horn (7) is subject to error because PBI is invalidated if the patient has recently received iodinated drugs. The free thyroxine index is now mostly calculated as the product of T_4 and triiodothyronine resin uptake (19, 23, 34, 37). The thyroxine-binding

capacity of TBP has also been assessed by using labelled thyroxine instead of triiodothyronine (6, 13, 22). That the free thyroxine index correlates closely with the free thyroxine concentration has been actually demonstrated (1, 22, 33, 37).

The present paper describes a modification of the competitive protein-binding technique for determination of T_4 and reports the clinical experience obtained with this method. The diagnostic value of three different free thyroxine indices was compared. One free thyroxine index was the product of T_4 and a triiodothyronine uptake test, and another the product of T_4 and a thyroxine uptake test. The original free thyroxine index was also calculated.

MATERIAL AND METHODS

Patients

The 330 patients studied comprised 106 hyperthyroid and 52 hypothyroid patients, 59 patients with non-toxic goitre, and a control group of 113 euthyroid patients with various non-thyroidal disorders.

The diagnosis of hyperthyroidism was based on clinical symptoms and signs as described by Lamberg et al. (18). In hypothyroidism the symptoms and signs looked for were those described by Ellervik et al. (5). In addition, the following laboratory tests were studied in both hyper- and hypothyroidism: PBI, T, triiodothyronine and thyroxine uptake by Sephadex (see later), absolute free thyroxine (21), serum cholesterol, urinary excretion of hydroxyproline radiolabelled tests (thyroid uptake), urinary excretion, protein-bound radiolabelled, and in some instances glucose-6-phosphate dehydrogenase activity in erythrocytes. Patients taking oral contraceptives or other drugs known to alter thyroid function tests (17) were excluded from the study. However patients with a history of iodine intake were accepted, but were not included in the study of PBI and the free thyroxine index based on PBI. The free thyroxine indices studied here were not taken into derivation and are calculated after making the final

Before treatment was started the preliminary diagnosis was made from all available data, and when symptoms and signs were not in conformity with laboratory test results the clinical evaluation was decisive. The final diagnosis was reached on re-examination of the patients for response to treatment for hyper- or hypothyroidism. The disappearance of all symptoms and signs or a definite improvement was required. Patients in whom the diagnosis could not be made beyond reasonable doubt were not included in the study. Of the hyperthyroid patients 81 had diffuse goitre and 45 nodular goitres as judged from palpation, thyroid scanning, and sometimes from the findings at operation (18). All hypothyroid patients had primary hypothyroidism.

The diagnosis of euthyroidism was based on clinical grounds with support from the laboratory tests mentioned above. All records were re-examined 1-3 years after the

preliminary diagnosis was made. When the course of the disease was not in conformity with euthyroidism the patient was not included in the study. As for hyper- and hypothyroidism, clinical evidence was always given priority when a discrepancy with laboratory test results existed. In hospital practice we considered it more valuable to use a control group of euthyroid patients than healthy subjects when assessing the diagnostic value of the laboratory tests studied. The euthyroid patients were hospitalized for various disorders, e.g. congestive heart failure, atrial fibrillation, hypertension, obesity, gastrointestinal complaints, and the thyroid evaluation was called for due to some symptom or sign suggesting a thyroid disease. Some of the euthyroid patients attended only the Out-patient Department. Several of the outpatients were sent for a thyroid check-up because of an elevated PBI, which was found to be due to ingestion of iodinated drugs.

Twenty patients with non-toxic diffuse and 39 with non-toxic nodular goitre were studied. The diagnosis of euthyroidism was reached as described for euthyroid patients. Some of these patients are operated on, some were treated with thyroxine, but many were only kept under observation and obtained no treatment.

It is assumed that, by this diagnostic procedure in which the course of the disease was evaluated before making the ultimate thyrometabolic diagnosis, the possible bias for putting patients into wrong categories of thyroid function due to the primary knowledge of laboratory data will be minimized.

Determination of thyroxine

The method for determination of thyroxine is a competitive protein-binding assay which utilizes the specific binding properties of TBO for thyroxine. A small amount of labelled thyroxine is added to a pooled serum. A basic assumption is that radiolabelled thyroxine is in equilibrium with unlabelled thyroxine and that the ratio of protein-bound and free radioactivity is equal to the ratio of protein-bound and free thyroxine in serum. A reagent used to separate bound and free thyroxine. When known amounts of thyroxine are added to the radiolabelled-TBO solution, the relative amount of bound radiolabelled thyroxine decreases and a standard curve is obtained. The thyroxine concentration of the unknown serum is determined from this standard curve.

The competitive protein-binding assay of T is fairly specific for thyroxine. In vitro addition of relatively large amounts of triiodothyronine, desiodothyronine, and diphenylhydantoin have an elevating effect on T (27). Physiological concentration of triiodothyronine is a negligible source of error. Only administration of triiodothyronine, diphenylhydantoin and possibly phenobarbital may sometimes increase the T value.

Thyroxine standard substance: L-thyroxine, free acid (Sigma, USA), is stored in a desiccator at 20°C for not longer than half a year. The stock standard solution containing 1.20 nmol thyroxine/l is prepared by adding 21.3 mg L-thyroxine to 2.5 ml propylene glycol (Fiedl., Germany); 4 ml 0.1 N NaOH is added during constant stirring until a clear solution is obtained. The final volume is adjusted to 25 ml with distilled water and stored at

-20°C for not more than 6 weeks. The working standard containing 12 µmol/l thyroxine is prepared by mixing 1.0 ml stock standard solution, 0.5 ml 0.1 N NaOH, and 1.0 ml propylene glycol in distilled water and adjusting the volume to 100 ml. This working standard is stored in refrigerator (4°C) and renewed every week. The dilute working standard solution, 10th thyroxine content of 120 nmol/l, is prepared from 1.0 ml working standard by adding 0.5 ml 0.1 N NaOH and adjusting the volume to 100 ml with 95% ethanol. This dilute working standard was renewed daily.

TBG-¹²⁵I-thyronine reagent. 7 ml fresh pooled normal human serum (or this serum stored at -20°C for not longer than 1 month), 2.5 ml 1% phenol (w/v), and 2.5 ml propylene glycol is mixed with 75 mM barbiturate buffer solution, 15 µl (5–8 µCi) carrier-free ¹²⁵I-thyronine solution (The Radiochemical Centre, England) is added to this solution, stored at 4°C and used for only 4 days.

Ion exchange resin. Rexton 202 anion exchange resin (Fisher Scientific Co., USA) is used. The resin is washed with 1.5 l distilled water three times and thereafter packed into chromatography columns and eluted with 75 mM barbiturate buffer until the pH of the eluate is 8.6 (about 5 l buffer is needed). The resin is dried at 80–90°C for 3 h on filter paper and then filtered through 0.7 µm pore filter.

Procedure. 0.5 ml patient serum is pipetted into glass tube and 1.0 ml 95% ethanol is added. Mixing done with Vortex mixer for 10 sec. The tube is allowed to stand for 10 min. After centrifugation at 2000 rpm for 10 min 300 µl of the supernatant is transferred to two plastic tubes. Of the dilute working standard, triplicate samples of 50, 100, 150, and 200 µl are pipetted into plastic tubes. All the tubes are evaporated to dryness at 45°C in water bath using stream of air. 30 min is needed for evaporation to dryness; 4 ml TBG-¹²⁵I-thyronine reagent is then added to each tube and these empty tubes. The rack of tubes is vigorously shaken manually for 30 sec and incubated for 8 min in 45°C water bath, and again shaken for 30 sec. The rack of tubes is placed into an ice-water bath, with sufficient water to reach above the solution level in the tubes, and kept in refrigerator. After 1 h the pan with the rack of tubes is removed from the refrigerator and 200 ± 15 µg of digoxigenin-stored resin is added to each tube with spoon. The rack is shaken mechanically for 6 min and then replaced into the ice-water bath. A 2 ml aliquot is drawn from each tube and radioactivity is counted in γ-counter for 1 min. The percentage bound radioactivity in each tube is calculated as follows: cpm in 2 ml aliquot/100 cpm in 2 ml TBG-¹²⁵I-thyronine reagent. After calculation of the percentage of bound radioactivity the thyroxine content of the unknown serum sample is read from the standard curve. The standard curve is obtained by plotting the percentage bound radioactivity against the thyroxine content of the standards.

Recovery. Ethanol extraction of thyroxine from TBP is incomplete. Recovery was studied by adding ¹²⁵I-thyronine to pooled normal serum, allowing it to equilibrate for 15 min, 0.5 ml serum then being extracted with 1.0 ml 95% ethanol. The recovery for ethanol extraction in our determinations is 77 ± 2% (mean ± SD). Murphy

Table 1. Alteration in the temperature of the incubation solution placed in room temperature

Time (min)	Temperature (°C)	
	Volume of incubate (1 ml)	(4 ml)
0	0	0
3	4.5	3
6	8	6
9	11.5	8

(26) has shown that there are no other sources of error than those due to the extraction when known amounts of unlabelled thyroxine are added to plasma supernatants.

Incubation temperature. It is generally accepted that low incubation temperature is necessary for obtaining good results with the competitive protein-binding assay (7). In all methods so far reported 1 ml radiothyronine-TBG reagent has been used. It was assumed that by using larger reagent volume more stable incubation temperature might be achieved. A standard alteration in incubation temperature after adding the resin to 1 and 4 ml radiothyronine-TBG reagent at 0°C. The tubes were allowed to stand for 9 min at room temperature while measuring the temperature of the incubation solution. A slower rise in temperature was observed with 4 ml than 1 ml reagent volume (Table 1).

Trisiodothyronine and thyroxine uptake tests

The trisiodothyronine uptake test (T_3U) as performed as described by Hansen (11), but using Sephadex G-25 coarse grain particles (Pharmacia, Sweden) and not medium grain, and ¹²⁵I-labelled trisiodothyronine instead of ¹²⁵I-labelled. In order to minimize the day-to-day variations and to increase the reproducibility of the method, we included pooled normal serum in all series analysed. The final result was expressed as percentage of this reference value. This test is basically similar to earlier tests utilizing the binding of labelled trisiodothyronine to erythrocytes or resin (10, 24).

The thyroxine uptake test (T_4U) used in this study has been described previously by Larvén et al. (22). The distribution of ¹²⁵I-thyroxine between TBP and Sephadex coarse grain particles was determined. A pooled normal serum was run as control in all series and the final result expressed as percentage of this reference value.

Calculation of free thyroxine indices

Three different thyroxine indices were calculated. One free thyroxine index—here called FTI_1 —was defined as the product of T and T_3U and for convenience multiplied by factor of 10^{-4} $FTI_1 = T \cdot T_3U \cdot 10^{-4}$. Another free thyroxine index (FTI_2) as calculated as the product of T and TU $FTI_2 = T \cdot TU \cdot 10^{-4}$. A free thyroxine index (FTI_3) similar to the original index of Clark and Horn (7) was obtained from the product of PBI , determined by the Technicon AutoAnalyzer, and T_3U $FTI_3 = PBI \cdot T_3U \cdot 10^{-4}$.

Table II. Thyroid function tests in various thyroid disorders (mean \pm S.D.)

	T		T U		T U		Free thyroxine index			
	$\mu\text{g}/100 \text{ ml}$	n	%	n	$\mu\text{g}/100 \text{ ml}$	n	T	T U	n	T T _U
Euthyroidism	7.3 ± 1.7	113	97 ± 15	109	96 ± 14	94	7.0 ± 1.6	109		7.0 ± 1.7
Toxic diffuse goitre	14.7 ± 3.6	61	155 ± 49	49	144 ± 30	41	23.9 ± 11.3	49		20.9 ± 8.4
Toxic nodular goitre	13.3 ± 3.0	45	134 ± 38	38	133 ± 37	37	17.8 ± 6.6	38		17.0 ± 6.6
Non-toxic diffuse goitre	8.5 ± 1.9	20	90 ± 13	19	93 ± 10	18	7.6 ± 1.6	19		7.8 ± 1.9
					NS		NS			NS
Non-toxic nodular goitre	8.3 ± 1.5	39	88 ± 13	27	93 ± 12	29	7.4 ± 1.2	37		7.8 ± 1.9
					NS		NS			NS
Hypothyroidism	2.9 ± 1.5	52	76 ± 15	50	74 ± 11	44	2.5 ± 1.3	50		2.3 ± 1.2

$p < 0.05$, $p < 0.01$ $p < 0.001$ NS = non-significant (Student's *t*-test).

RESULTS

Methodological aspects

The recovery of thyroxine was equal to that reported in the original methods (26, 27). It was extremely important that the evaporation in dryness was complete, otherwise falsely elevated T_4 values were obtained. We found that the thyroxine standard solutions could not be stored for a lengthy time. We used the standard solutions for a much shorter time than Murphy (26). Our storage times are more in line with those recommended by Nobel and Barnhart (30). Serum used for preparing the TBG-radiothyroxine reagent must be stored in a deep-freezer and thawed immediately before use. If the serum was kept at refrigerator temperature for more than 2 days,

the standard curve became unsatisfactory especially for reading low values of thyroxine.

The major modification introduced was the use of 4 ml TBG-radiothyroxine reagent instead of the usual 1 ml. It is our experience that the use of 1 ml reagent often raises serious difficulties at the end of the analytical procedure. The reason was probably the rapid changes in temperature after adding the resin. A rise in temperature will cause a shift in the equilibrium of bound and free thyroxine in favour of the free fraction. Free thyroxine will be bound irreversibly to the resin and this will further initiate a release of bound hormone. A prerequisite for getting reproducible T_4 results is that all analytical conditions, including temperature are equal for all samples tested. When the rack of test tubes is removed from the ice-water tray the temperature of the incubation solution rises and the rise was found to be faster when using 1 ml reagent than 4 ml. The slower rise in temperature is of special importance when analysing a large number of serum samples.

The problem of keeping a constant low temperature was probably observed also by Sierstahl-Nielsen (35) because only eight samples at a time were included in the final step of analysis. In addition to loss of time, we still had difficulties in obtaining good reproducibility when performing the T_4 determination in this way. The increase in T_4 for the last samples was often about 20%. When analysing as many as 50 samples at a

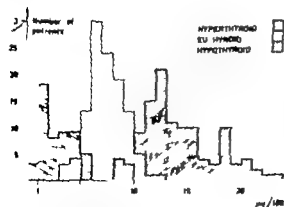


Fig. 1 T_4 determined by competitive protein-binding techniques in co-hyper and hypothyroid subjects.

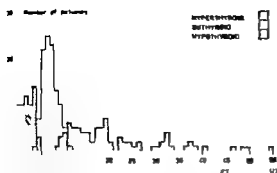


Fig. 2 FTI calculated as the product of T and thyroxine uptake test (TU) in eu-, hyper and hypothyroid subjects.

time an erroneous increase of up to 50% in T for the last samples was sometimes found. With the present modification using 4 ml TBG-radio-thyroxine reagent, no significant increase in T at the end of a series containing 50 tubes was encountered.

In order to test the reproducibility of the method, a pooled normal serum was analyzed nine times over a period of two weeks. The result expressed in terms of mean \pm SD was 7.8 ± 0.7 μ g/100 ml. Duplicate samples were analysed. The SD of the difference between duplicate determinations in the euthyroid range was ± 0.41 μ g/100 ml.

Clinical data

In euthyroid subjects the mean T was 7.3 μ g/100 ml uncorrected for recovery (Table II). As can be seen from Fig. 1 the euthyroid control values approximately met the requirements of a Gaussian distribution, and the control range of values was therefore defined as the mean ± 2 SD equal to 3.9–10.7 μ g/100 ml. The same type of distribution of euthyroid values of the two free thyroxine indices based on T was found (Figs. 2 and 3). The control range of values for FTI was 3.8–10.2, and for FTI 3.6–10.4.

In both diffuse and toxic nodular goitre T and the free thyroxine indices were significantly higher than the corresponding control values. When both groups were combined, 94% of hyperthyroid patients had T_4 values within the normal range (Fig. 1). In hyperthyroidism 4.6% of the FTI values and 6.4% of the FTI values were within the corresponding control ranges (Figs. 2 and 3).

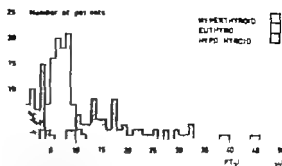


Fig. 3 FTI calculated as the product of T and triiodothyronine uptake test (T_3U) in eu-, hyper and hypothyroid subjects.

Of the 52 hypothyroid patients 31% had T_4 values within the normal limits for euthyroid subjects (Fig. 1). In hypothyroidism 8.0% of the FTI and 9.1% of the FTI values were within the corresponding control ranges (Figs. 2 and 3).

Patients with non-toxic diffuse and non-toxic nodular goitre had significantly raised T values. In these patients the TU and T_3U values were somewhat lower than the corresponding control values and therefore, the mean free thyroxine indices were not significantly increased.

The correlation between TU and T_3U was studied and is presented graphically in Fig. 4. When tested statistically the correlation was found

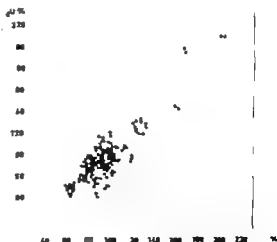


Fig. 4 Relationship between thyroxine Sephadex-uptake test (TU) and triiodothyronine Sephadex-uptake test (T_3U). The linear correlation between the two tests is described by the estimating equation $TU = 1.03 (T_3U) - 1.92$ ($r = 0.87$ $p < 0.001$).

to be highly significant (correlation coefficient $r=0.87$ $p<0.001$) corresponding to an estimating equation of $T_4U=1.03 (T_3U)-1.92$.

PBI was determined in the eu- hyper and hypothyroid patients with no history of recent ingestion of iodinated compounds. The distribution of PBI and T_3I values in the euthyroid group was skewed in the direction of high values. The end-points of the control range 2.5 and 97.5 percentiles were, therefore, interpolated from the ordered samples. For PBI the control range in 71 subjects was 3.4–12.8 $\mu\text{g}/100\text{ ml}$, and for T_3I in 70 subjects 3.3–11.8. In 51 hyperthyroid patients 39% of the PBI values were within the control range and in 47 hyperthyroid patients 17% of the T_3I values were within the corresponding control range. In 35 hypothyroid patients 17% of the PBI values were within these 95% limits, and in 33 hypothyroid patients 12% of the T_3I values were within the control range defined in this way. The correlation coefficient between T_3I and FT_4I was $r=0.93$ ($p<0.001$) and between T_3I and FT_3I , $r=0.96$ ($p<0.001$).

DISCUSSION

When working with the competitive protein-binding T_4 assay of Murphy (26) we found several of the modifications made by Nobel and Barnhart (30) to be useful. Nevertheless we considered some further modifications necessary when large series of serum samples were analysed. The need for a constant temperature near to 0°C in the final step of analysis was given careful attention. An elevating effect on T_4 values to an increase in temperature has not previously been stressed. Also the importance of using fresh serum for preparing the TBG-radio-thyroxine reagent and short storage times for the thyroxine standard solutions is emphasized.

In euthyroid subjects the mean T_4 value uncorrected for recovery was very similar to that obtained with competitive protein-binding techniques by others (2, 5, 12, 19, 34). The degree of diagnostic discrimination achieved with T_4 in both hyper and hypothyroidism was similar to that reported from this laboratory for a smaller patient material (22) but less than that reported by some other investigators (5, 33). Murphy et al. (28) reached an overall compatibility with the

final diagnosis in 97% but in their study patients using drugs known to alter T_4 were meticulously excluded. This was not possible in the same extent in our material, which comprised many out-patients. There were also many patients with mild hyper and hypothyroidism included in this study.

Our results are in some respects in agreement with those reported by Lee et al. (19) as regards hypothyroidism. It seems evident that there are patients with low-normal T_4 who benefit by replacement therapy. In these patients with mild hypothyroidism laboratory confirmation can be reached more reliably with the free thyroxine indices, but even these tests sometimes fail. In this situation the diagnosis must be derived from symptoms and signs and from a trial with substitution therapy. Preliminary observations in this laboratory indicate that the determination of thyrotrophin in blood is often of value in the diagnosis of mild hypothyroidism.

That patients with non-toxic nodular goitre have moderately elevated PBI and T_4 levels and slightly raised free thyroxine concentration has been reported previously from this laboratory (21, 22). Similar results were now obtained in non-toxic diffuse goitre. Because both PBI and T_4 were increased in non-toxic goitrous subjects the increase cannot be due to non-hormonal iodinated compounds. However the small increase in mean free thyroxine index, which correlates closely with the free thyroxine concentration, was not statistically significant.

PBI was very much less efficient than T_4 in distinguishing between euthyroid and hyperthyroid subjects. Spuriously elevated PBI values were often encountered and it is most probable that in several instances an intake of iodinated drugs was not revealed by the patient's history. This also affected T_3I , which was less accurate than the two other free thyroxine indices. The skew distribution of PBI values in euthyroid patients of the present study is strong evidence of undetected iodine contamination because in a previous investigation the PBI values in euthyroid control subjects were normally distributed (18). Also, because T_4 values had a normal distribution, it is improbable that the skew distribution of PBI values comes from the use of euthyroid patients and not healthy subjects as a control group. The difficulty of ruling out iodine contamination

tion is apparent and clearly demonstrates the need for routine T_4 assays in clinical practice.

In hypothyroidism PBI was more accurate than T_4 . One reason is probably the better precision of the PBI determination. The diagnostic value of all free thyroxine indices differed only but in hypothyroidism.

Mitchell et al. (24, 25) used both labelled thyroxine and triiodothyronine in their resin uptake tests and obtained comparable results with the two methods. The correlation coefficient $r=0.89$ between the thyroxine and triiodothyronine resin uptake tests of MacLagan and Howorth (23) is very similar to that found in the present study using different techniques. Despite the excellent correlation when a large number of values from eu-hyper and hypothyroid patients were compared, we found that an increased T_4U is not always accompanied by an increased T_3U or the reverse. In the present methods dextran particles were substituted for ion-exchange resin. What

is perhaps more important, phosphate buffer is used instead of barbiturate buffer. When barbiturate buffer is used, binding of thyroxine to thyroxine-binding prealbumin (TBPA) is inhibited, and thyroxine is bound only to TBG and albumin (15). In phosphate buffer there is also binding to TBPA. Triiodothyronine is bound only to TBG and albumin in both these buffers, and with binding characteristics different from those of thyroxine. TBG is the major TBP but TBPA is also considered to be of some physiological importance. Binding of thyroxine and triiodothyronine to albumin is probably without physiological significance. Despite this theoretical difference between the T_4U and T_3U tests the two free thyroxine indices based on these tests were of approximately equal diagnostic accuracy.

Even though FTI gave a somewhat better diagnostic discrimination for both hyper and hypothyroidism than FT $_4$, none of these free thyroxine indices should be dismissed from the thyroid laboratory arsenal. The reason is that in both hyper and hypothyroidism we found in several instances that, when one free thyroxine index was within the normal range, the other

is in conformity with the ultimate diagnosis. It is therefore recommended that both of these free thyroxine indices are determined for corroboration of the diagnosis of mild thyrometabolic disease.

ACKNOWLEDGEMENTS

This study was aided by grants from the National Research Council for Medical Sciences, the Sigrid Juselius Foundation, the Medical Relief Fund Liv och Hälsa and the Nordic Insulin Fund.

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SUCCESSFULLY TREATED PRIAPISM IN ACUTE MYELOBLASTIC LEUKEMIA COMPLICATING HODGKIN'S DISEASE

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Abstract. Acute myeloblastic leukemia developed in a 22-year-old male patient who had suffered from Hodgkin's disease since his eighth year. In this period of 14 years the patient received radiotherapy (a total of 14 000 rads) and several courses of cytotoxic drugs. During the final admission priapism and bilateral central retinal vein thrombosis developed. Both complications subsided during combined treatment with cytotoxic drugs and the defibrinating agent anecrod. After 34 days of treatment priapism gradually diminished and eventually sexual function was restored. Notwithstanding serious thrombocytopenia, lowering of the plasma fibrinogen level below 50 mg/100 ml during most of the 60 days of anecrod treatment did not cause hemorrhagic complications.

Priapism is a rare but dramatic complication of leukemia. The causal relationship is not clear. Conceivably the high number of circulating leukemic cells increase blood viscosity leading to stasis of the blood stream. Another possibility is leukemic infiltration of the venous drainage of the penis. Furthermore neurogenic and psychogenic factors may play a role. But, whatever the initial cause, thrombosis in the venous drainage area of the corpora cavernosa is a final event in persistent priapism. Treatment with heparin or coumarin derivatives is notably unsuccessful and the use of thrombolytic (5) and defibrinating (1) agents has been recommended.

We recently treated a patient with Hodgkin's disease of 14 years duration in whom acute myeloblastic leukemia complicated by priapism and bilateral central retinal vein thrombosis developed. Both complications subsided during treatment with the defibrinating agent anecrod (Arvin, registered trade mark of Wyford Laboratories Ltd, London) combined with antileukemic drugs.

CASE REPORT

A 22-year-old male patient had been in good health until the age of 8, when Hodgkin's disease was diagnosed. The

most important clinical data from 1957 to 1971 are summarized in Table I. The patient was remarkably well adjusted. He worked in an office and was finishing secondary school in evening classes. In Sept. 1971 radiotherapy was given because of enlargement of cervical and mediastinal lymph nodes; the liver was not palpable. During this admission a remarkable blood picture was found before irradiation: the leucocyte count was 50 000/mm³ with 70% blasts. Hb as 12.4 g/100 ml and platelets 71 000/mm³. After radiotherapy the leucocyte concentration fell to 16 000/mm³ with 40% blasts.

On Nov. 14, 1971, the patient was admitted again because of fever (40°C) without signs or symptoms of local infection. Several blood cultures remained negative. The liver was found to be enlarged and there were only a few small cervical and inguinal lymph nodes. Several teeth were carious. No other important abnormalities were found on physical examination. Hb had decreased to 8.9 g/100 ml, WBC was 300 000/mm³ with more than 95% myeloblasts, platelets were 55 000/mm³. A strongly positive peroxidase reaction of the circulating blasts confirmed the diagnosis of acute myeloblastic leukemia.

The patient was to be married on Nov. 18 and it was decided to hold the marriage in the hospital. On the morning of the wedding day however priapism developed in association with diminished visual acuity. Examination of the fundus showed bilateral central retinal vein thrombosis. The plasma creatinine level was increased compared to former admissions. No laboratory evidence of disseminated intravascular coagulation was obtained (normal thrombin clotting time, negative ethanol gelation test, normal levels of fibrinogen and factor V).

Leukemia treatment consisted of repeated 5-day courses of daunorubicin (1.5 mg/kg b.wt.) by i.v. injection in fast-flowing drip on the first day and cytosine arabinoside (1 mg/kg b.wt.) by daily i.v. injections on days 1-5 inclusive. Each course was separated by five days (Fig. 1). Packed red cells were given as needed. Platelet concentrates were infused whenever petechiae appeared spontaneously.

Priapism was initially treated with L-heparin for three days without any success. On Nov. 20 treatment with anecrod (Arvin) was started. Because of the low number of platelets, relatively low induction dose was administered intravenously (1.5 U/kg). In less than 9 hours the fibrinogen level decreased to values lower than 100 mg/100 ml

considered to be absolutely contraindicated for our patient. Recent reports have claimed success with anecrod therapy for priapism (1) and for central retinal vein thrombosis (3). Anecrod, a purified anticoagulant fraction of venom of the Malayan pit viper causes disappearance of plasma fibrinogen by splitting off fibrinopeptide A, the resulting fibrin monomers are removed from the circulation without apparent untoward effects. No effects on other coagulation factors have been observed and defibrination by means of anecrod is remarkably free of hemorrhagic side-effects (2), although high levels of fibrin(ogen) degradation products during the first few days of treatment might inhibit platelet function. The exact mode of action of anecrod on thrombi *in vivo* is unknown. Conceivably the removal of fibrinogen from the circulation may allow local fibrinolytic mechanisms to resolve the thrombus without a fresh thrombus being formed (1). Although thrombocytopenia is also considered to be a contraindication for anecrod therapy (2), we decided to try anecrod, because other therapeutic measures such as streptokinase and surgical intervention seemed to be out of the question. We could find no reports on the use of anecrod therapy in patients with thrombocytopenia. For that reason a low starting dose of 1.5 U/kg was given, after which the dose was gradually increased during the following ten days. Continuous infusion produced a more stable effect on plasma fibrinogen than administration of the same daily dose at 12-hour intervals (Fig. 1).

In the first few weeks of anecrod therapy central retinal vein occlusion improved, as did glomerular filtration rate although the leucocyte count was still higher than 100 000/mm³. However priapism only improved when cytotoxic therapy had used a fall in the leucocyte count to about 6 000/mm³ and anecrod had lowered plasma fibrinogen to practically zero. For this reason it is

impossible to decide which part of the treatment was ultimately responsible for the return to normal size and function of the penis.

Resistance to therapy has been claimed in 2 of 18 patients with thromboembolic disease treated with anecrod intravenously for periods up to three weeks (6). In our patient anecrod maintained its defibrinating activity during the whole 60-day course. Apparently no antibodies were formed, which is probably related to immunodeficiency caused by the leukemic process or concomitant cytotoxic drug treatment.

Most striking was the absence of hemorrhagic complications. Even extraction of three molar teeth did not cause abnormal bleeding. In our opinion this confirms the claim that anecrod is a relatively safe drug and shows that in a desperate situation it is justified to administer anecrod even in the presence of thrombocytopenia, provided no spontaneous bleeding exists.

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EDITORIAL

FAMILIAL LCAT DEFICIENCY

Familial lecithin cholesterol acyltransferase (LCAT) deficiency is a disease characterized by diffuse corneal opacities, normochromic anemia with decreased erythrocyte life span, proteinuria with late renal insufficiency and in most patients turbid or milky plasma. Foam cells are seen in the bone marrow and in the kidney glomeruli and "sea blue histiocytes" are detected both in bone marrow and spleen.

Familial LCAT deficiency was first described in 1967 (family I) in three Norwegian sisters, who were then 20, 32 and 34 years old (4, 5, 14). The plasma of the two older ones was turbid or milky with triglyceride concentration above normal. Elevated plasma concentrations of free cholesterol and lecithin and elevated levels of plasma lysolipids and cholesteryl ester were found in all. The enzyme LCAT could not be detected in their plasma. Smears of peripheral blood showed many erythrocytes of the target cell type. Stages of their red cell lipids revealed marked increase both in erythrocyte cholesterol and lecithin content (6). In decrease in phosphatidyl ethanolamine and sphingomyelin. In 1969 Swedish family (family II, Table I) with sister and brother was described (9). The brother died in uremia at the age of 40 years. He had similar corneal opacities to those of his sister, anemia, proteinuria and lipemic plasma had also been observed.

A third family (family III) from the same north-western coastal area of Norway as family I was described in 1970 (11). A sister (44 years) and brother (38 years) both had typical corneal changes, anemia, proteinuria and lipemic plasma. Their renal function was normal.

In June 1973 Dr A. Skårhøj of Ålesund brought to our attention fourth family (family IV). Both the sister (50 years) and the brother (55 years) living in the same western coastal area of Norway as families I and III had low total cholesterol, almost all of it being in the free form, and only moderately elevated triglycerides in plasma. The brother only had proteinuria and renal insufficiency. They are the oldest known living patients with this disease. Two additional brothers from Sørhøland in the same area combined with LCAT deficiency have also been reported by Uttermann et al. (18).

All the living Scandinavian patients with this disease, reported so far, have been studied in our department—the first three sisters for 7 years up till now. It may be proper to give survey of the disease based on personal clinical observations and experience, since a series of interesting clinical and chemical aspects seem to be attached to this disease.

Plasma lipids

The plasma has slightly turbid or milky white appearance. Cholesteryl ester and lysolipid concentration are decreased in all, and free cholesterol and lecithin is increased (Table II). The concentration of total plasma cholesterol is low in the two afflicted members of family IV normal in one and high in the others. Plasma triglycerides have been elevated in all but two.

The cholesteryl esters in plasma are believed to be derived from chylomicrons, since cholesteryl esters become labelled after intake of radioactively cholesterol, but not after plasma infusion. Injection of radioactive mevalonate (14). Furthermore most of the cholesteryl esters are present in the very low density lipoproteins, and contain large amounts of oleic and palmitic, as do the chylomicrons in thoracic duct lymph.

Plasma lipoproteins

Qualitative abnormalities in the apo-lipoproteins are not present since apo-A I and II, apo-B, apo-C I, II and III, and also apo-D (thin line) have been demonstrated in LCAT-deficient plasma (12). Free cholesterol and lecithin are present in increased amounts, both in the very low and high density lipoproteins (VLDL, LDL, HDL) of the patients. The ratio of cholesteryl ester to free cholesterol decreases rather than increases with increasing lipoprotein density (8, 15). α -lipoproteins were originally believed to be absent, since they were not seen in cellulose acetate strip electrophoresis. As later shown by Torvik (16) α -lipoproteins are present, apo-A however being quantitatively reduced to about one-third of the normal concentration.

HDL in LCAT deficiency have been found to contain two subfractions on gel filtration with Sephadex G-200 (1, 15). One major large-molecular weight fraction is rich in free cholesterol and lecithin, migrates as an α -globulin, contains disc-shaped structures with diameter of 190-200 Å and appears mainly in stacks of rouleaux formation. The other fraction of smaller molecular weight is close to normal in its relative content of free cholesterol and lecithin, migrates electrophoretically as albumin and is made up of structures 45-60 Å in diameter.

LDL are also heterogeneous (1, 8, 15). The concentration of apo-lipoprotein B is quantitatively reduced in all, but in varying degree (12). Three subfractions of LDL are found on gel filtration with 2% agarose. Subfraction 1 (LM-LDL) emerges with the void volume, is rich in free cholesterol, lecithin and triglycerides and is the electron

Table I. Clinical data on nine patients with familial LCAT deficiency

Pat. no.	Sex	Born	Corneal opacity noted	Proteinuria from	Anemia detected	General health
<i>Family I</i>						
1	♀	1933	c. 1948	1952	1952	From 1966 asthenia and edema. 1969/70 progressive renal failure. Hemodialyses from April-70. Jan. 73 transplantation. Died July-73
2	♀	1935	At puberty	1954	1954	Frequent infections, otherwise healthy
3	♀	1947	c. 1962	1964	1965	Frequent cystitis and tonsillitis, otherwise healthy
<i>Family II</i>						
4	♀	1921	In childhood	1925	1963	Some asthenic complaints. Frequent cystitis and common colds
5	♂	1924	1958	1941	1961	Died in anemia 1964
<i>Family III</i>						
6	♀	1926	1966	1958	1958	Healthy
7	♂	1932	1962	1950	1962	Healthy
<i>Family IV</i>						
8	♂	1918	In childhood	1943	1956	Rheumatismus acutus 1948. From 1972 progressing renal insufficiency
9	♀	1913	In childhood	Not present	I childhood	Healthy

microscope is seen to contain large flattened structures 900-1200 Å in diameter. Subfraction II has a lipid composition identical to that of lipoprotein X (LPX) (17). It does not react with antiserum to normal LDL in agar gel double diffusion experiments, but with antibodies to apo-lipoproteins C and, after delipidation, also to antiserum to albumin. Subfraction II is therefore clearly related to LPX. On agar immunoelectrophoresis of serum from the patients, cathodal migration of an abnormal lipoprotein is seen. Subfraction III emerges in the same position as normal LDL, looks similar to normal LDL in the electron microscope but contains more triglycerides.

Table II Plasma lipids (mg/100 ml) in eight patients with familial LCAT deficiency

Pat. no.	Age (y)	Cholesterol		Tri-glycerides	Phospho-lipids
		Total	Free		
1	33	302	292	312	375
2	31	565	515	573	610
3	20	143	134	129	165
4	47	369	271	533	380
6	44	215	184	900	318
7	38	235	204	630	374
8	55	133	129	251	116
9	60	107	105	105	138

VLDL are also abnormal and migrate electrophoretically as β -lipoproteins.

The most striking abnormalities of the plasma lipid and lipoprotein composition thus described in the absence of LCAT activity are the very low content of cholesterol ester and lysolecithin, the quantitative reduction in the concentrations of apo-lipoproteins A and B and the appearance of LPX. Signs of cholestasis are not present in any of the patients. Bile acids are normal in concentration and pattern in plasma as well as in bile. LPX has hitherto been recognized as specific for cholestasis. The findings in patients with LCAT deficiency show that this is not the case, since LPX is demonstrated in all of them. Both of these conditions are characterized by the occurrence of large amounts of plasma free cholesterol and lecithin. Possibly this may lead to the formation of LPX in circulating plasma, but LCAT may also have a physiological role in the breakdown of LPX.

With the techniques available at present the estimation of LCAT activity is highly specialized investigation. The LPX test may therefore be used as screening procedure for the detection of LCAT deficiency.

Lipid deposits in LCAT deficiency

Corneal opacity is the easily detectable and early occurring clinical sign in all patients with familial LCAT deficiency (2), and this finding should be a clue to the diagnosis. The corneal opacities are of the suns type in all patients, but slight differences in the amount of

opacity has been noted, being most pronounced in the eldest of the three Norwegian sisters. Examination of cornea reveals in all patients nebulous cloudiness and pronounced opacity of annular shape near limbus resembling marked arcus lipoides. The corneal surface is regular and brilliant with normal curvature and stromal thickness. The opacities are localized to the parenchyma and composed of innumerable minute grayish dots, abundant in the papillary zone and evenly distributed in all layers of the stroma. The arcus is separated from limbus by narrow relatively clear zone of corneal tissue and close to the limbus all stromal dots vanish. It seems reasonable to believe that the corneal opacity represents deposits of some lipid, the nature of which has not been clarified as yet.

Retinal changes

Patient 1 (Table I) had normal vision until May 1971, but from then on gradual reduction occurred in visual acuity and fields. The left eye suddenly turned anisotropic in Dec. 1972. The disc margins were found to be completely blurred, its protrusion of 2-3 diopters, and some crystalline formations or seen within the papillary zones indicating deposits of pathological material rather than true papilledema. The retinal changes observed with disc protrusion and rupture of Bruch's membrane are most likely due to deposition of pathological lipid material (Härvén, Egge and Gjone, to be published). Lipid deposits in nervous tissue has been observed in many other dyslipoproteinemias like α - β -lipoproteinemia and in Tangier disease. The papillary changes seen in 16 of the patients with LCAT deficiency show that deposits in nervous tissue may occur also in this disease as it progresses.

Erythrocyte studies

Glover (7) proposed that LCAT is of importance for the homeostasis of free cholesterol in plasma membranes of peripheral tissues. Free cholesterol is taken up from plasma membranes into the circulating HDL, where it is esterified and transported to the liver for further conversion and excretion to bile acids. The studies of erythrocyte lipid composition in familial LCAT deficiency lend support to this theory.

Anemia is characteristic of the disease and in all patients is moderate and normochromic. Peripheral blood and bone marrow contains increased numbers of target cells. Rb being normal on starch gel electrophoresis in the Scandinavian patients. The erythrocyte Rf span is reduced to 16 and 17 days in two patients (5, 9). The results in LCAT-deficient patients is most likely due to moderate hemolysis combined with some reduction of compensatory increase in the red cell production (5).

Erythrocyte lipid composition is abnormal in all patients (6). Free cholesterol is increased up to twice the normal. Total phospholipids are normal, lecithin being markedly increased with concomitant decrease of phosphatidyl ethanolamine and sphingomyelin. The phosphatidyl seric content is remarkably constant in the red cells. The fatty acid composition of the phospholipids is abnormal, with increase of linoleic acid and decrease of arachidonic and very long chain fatty acid (6). These

findings are compatible with the theory that LCAT controls the composition of the lipids in the red cell membranes, as are the findings of the reversibility of these changes. Erythrocytes from the patients incubated with normal plasma have reduced cholesterol content. Normal erythrocytes become enriched in cholesterol when incubated in plasma from LCAT-deficient patients. Platelet lipids are normal both as regards lipid composition and fatty acid pattern.

Sea-blue histiocytes

The so-called "sea-blue histiocytes" have previously been found in various types of diseases, mostly connected with liver diseases and thrombocytopoenia IV have found them in all Norwegian patients with familial LCAT deficiency (11). The granules in the histiocytes are seen in the electron microscope to be composed of membranes in lamellar arrangement. In the cytoplasm they are seen packed like onion rings. The lamellar arrangement of membranes in the granules indicates that they are composed of phospholipids, and we have proposed that they may represent uptake of phospholipid-cholesterol containing membranous material circulating in plasma. Familial LCAT deficiency seems to be the only disease so far described in which all patients studied reveal the presence of "sea-blue histiocytes". Foam cells are also present in the bone marrow of most of the patients.

Lipid deposits in kidneys

Proteinuria has been present in all patients but one described so far. The male patient in family II died at 40 years of age in uremia. The oldest of the three sisters in family I rapidly developed renal failure at the age of 37 years. Kidney transplantation was performed in this case, but she died 6 months later. Also the male patient (55 years) in family IV has newly discovered and rapidly developing renal insufficiency, whereas his 60-year-old sister has normal urine and renal function. Proteinuria is therefore, with one exception, constant finding in patients with familial LCAT deficiency and renal failure is the serious life-threatening complication. LCAT deficiency should therefore be borne in mind in all cases of familial renal disease. Studies of the lipid deposits in the kidneys has been performed both by needle biopsy in some patients (5) and on the kidneys from bilateral nephrectomy of patient 1 in family I (10).

Light microscopical examination revealed that the Bowman's capsule is thickened, the mesangium prominent, and foam cells present in the glomerular tufts. Subendothelial deposits of lipid material are observed in the renal arteries and arterioles. Electron microscopical examination (10) showed that the capillary lumina are partly filled with meshwork of membranes and particles with an amorphous mottled structure. The capillary all as seen to be abnormal with fenestrated or lacking endothelium, irregular thickness of the basal membrane and fusion of the foot processes. The material seen in the glomeruli and in the arteries and veins is believed to correspond to membranous structures observed in negatively stained plasma specimens. Similar structures are seen also around the glomeruli and small vessels in liver biopsies from the patients. These lipids may represent

deposits of circulating abnormal lipoproteins rich in cholesterol and phospholipids, and correspond probably to the abnormal LM LDL described in plasma.

Atherosclerosis

Patients with familial LCAT deficiency seem to develop atherosclerosis. The observed changes in the renal arteries and arterioles are compatible with early atherosclerotic changes. The autopsy findings in the patient in family II who died at 40 years of age showed marked atherosclerosis in the aorta. Furthermore patient I in family I developed calcification in the aorta before the age of 40, and postmortem examination showed marked atherosclerosis of aorta and large arteries. These patients had extremely low concentrations of plasma cholesterol esters. They furthermore had subnormal quantities of apo-lipoproteins B. Even so, heterologously enough, they developed early atherosclerosis.

Aspects of treatment

Plasma infusions have been given in the attempt to change plasma lipids and lipoproteins towards a more normal pattern and to cause some reversal of the erythrocyte lipid abnormalities. Clinical effects of importance have not been observed, however.

Dietary manipulations have also been carried out. On a fat-free diet the concentration of the abnormal LM-LDL, rich in unesterified cholesterol and lecithin, decreased more than tenfold and that of the plasma triglycerides by one-third. Hypercaloric fat-free diet decreased the concentration of the LM-LDL even further whereas plasma triglycerides regained the original level. These results suggest that the LM-LDL fraction originates from chylomicrons, the primary form of transport of absorbed dietary fat. We further think that the accumulation of the LM-LDL is directly related to the LCAT deficiency. Whether fat-restricted diet can prevent the serious kidney complications is so far unknown. This disease may however be added to the list of inborn errors of metabolism, for which a dietary change may have a beneficial clinical effect in preventing organ deposits.

Kidney transplantation has been performed in one patient and may also be carried out in others. The LCAT activity in the plasma was not increased permanently by the transplantation. Calculations from the determinations of LCAT activity in connection with transplantation and massive blood transfusion showed that the half-life of LCAT in plasma was about 4 days.

CONCLUSIONS

Considerable biochemical information has been obtained and interesting clinical aspects have emerged from the studies of the patients with LCAT deficiency. The plasma lipid abnormalities seem to be the exception ones. The pathogenesis of many of the lipoprotein alterations, such as the heterogeneity of HDL and LDL, and the quantitative reduction of the apo-lipoproteins A and B are still unexplained. The occurrence of LP X in familial LCAT deficiency may be used as simple screening test for its diagnosis. Future studies employing purified LCAT may supply us with more complete information on the physiological role of LCAT in lipid metabolism.

The lipid deposits in LCAT deficiency have a diagnostic importance with respect to the corneal opacities. Also the phagocytosis of abnormal lipid material by histiocytes in particular staining properties ("sea-blue histiocytes") has diagnostic relevance. From a biochemical viewpoint the reversibility of the lipid changes of the erythrocytes has relevance for the physiological role of LCAT in the homeostasis of plasma membrane lipids. The lipid deposits in the kidneys represent the most important and life-threatening complication from the patient's point of view. Also in this metabolic disease dietary measures may be advisable. Why these patients with their lack of plasma cholesterol esters and low levels of apo-lipoproteins B develop atherosclerosis remains to be explained.

Further studies, particularly of the oldest patients with subnormal concentrations of total plasma cholesterol, one of whom has normal urine and kidney function, may give additional information on the prognosis of the disease.

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PLASMA LEVELS OF INDIVIDUAL FREE FATTY ACIDS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract. Plasma levels of individual free fatty acids (FFA) have been determined in 74 patients with acute myocardial infarction on admission and repeatedly during the first 24 hours at the hospital. Thirty-six patients admitted on the same criteria but in whom the diagnosis of myocardial infarction was later discarded served as control group. There were no differences in the plasma FFA levels between the two groups. The intrasubject variation in plasma FFA during the first day was large, the mean difference between the highest and lowest value in each individual being 50% of the level on admission. There was a general tendency for the FFA concentration to be higher in the more severely ill patients. No association between high plasma FFA levels and arrhythmias was observed. On the contrary the three patients who developed ventricular tachycardia had exceptionally low FFA levels before the occurrence of arrhythmia.

An elevation of plasma free fatty acids (FFA) after an acute myocardial infarction (AMI) was first reported by Kurien and Oliver (6) and later confirmed by several investigators (2, 5, 12). Different opinions have been expressed about the significance of this discovery especially with regard to the occurrence of serious arrhythmias. An increased prevalence of arrhythmias and disorders of conduction in patients with AMI with FFA values above 1200 μ moles/l was reported by Oliver et al. (10). A positive relationship between high FFA values and serious arrhythmias after myocardial infarction was also found by Gupta et al. (2) and by Reimann and Schwandt (12), but has been denied by others (9, 14, 15).

The question whether the high frequency of arrhythmias observed in some investigations is due to raised catecholamines or to the FFA themselves has also been the subject of controversy. From experiments in which extremely high FFA values were induced on dogs with myocardial in-

faction by administration of heparin and Intra-lipid® Kurien et al. (7, 8) asserted that the FFA themselves are arrhythmogenic. Others have, however, failed to confirm these findings (11). Since heparin causes an elevation of plasma FFA, Kurien et al. (7) have issued a warning against the use of this drug in patients with myocardial infarction. Nelson (9) and Russo et al. (13) found a rise in FFA after administration of heparin to patients with AMI, but no evidence of an increase in ventricular arrhythmias.

The aim of the present study was to obtain further information on this subject by analysing the levels of individual FFA and also by analysing FFA repeatedly during the first 24 hours after admission to obtain some information about the levels of individual FFA in the period immediately prior to serious arrhythmias.

MATERIAL AND METHODS

From March to May 1971 79 patients were admitted to the Coronary Care Unit at Serafimerlasarettet, Stockholm. All the patients admitted fulfilled at least one of the following criteria: 1) Central chest pain lasting for more than 15 min with onset within the previous 48 hours. 2) Frank pulmonary oedema without previously known valvular lesion. 3) Shock without suspicion of acute hypovolaemia or intoxication. 4) Syncope with ECG evidence of AMI. 5) Intractable angina pectoris.

A complete series of FFA analyses was carried out in 63 of the patients. In 15 patients the analyses are not complete due to admission on Saturday or Sunday and one patient died before any blood was collected. Of the 48 patients with a complete series of FFA analyses, 3 had suspected AMI and were excluded from the material.

4 had an AMI and in 36 the diagnosis was discarded; they served as control group. Twenty-seven out of the 36 control patients had angina pectoris and 11 of these had had myocardial infarction. The final diagnoses in

Table 1 Age and sex distribution in the patients

	N	Age (y)	
		Mean	Range
AMI			
Males	18	63	42-85
Females	6	73	63-84
Total	24	67	42-85
Controls			
Males	20	65	45-88
Females	16	71	54-88
Total	36	67	45-88

the other 9 controls were bronchopneumonia, cerebral embolus, paroxysmal atrricular fibrillation, Wolf-Parkinson-White syndrome, digoxin intoxication, myeloma, cerebral rhinopathy, syncope and atherosclerosis. Frank pulmonary oedema occurred in 3 of the control patients and in another 4 basal rates are noted on physical examination.

The diagnosis of AMI was based upon fulfilment of the following criteria. (a) ECG: appearance of a pathological Q wave and/or appearance or disappearance of a localized ST elevation followed by a T inversion in two or more of the 12 leads. (b) Serum enzymes: an increase in both GOT and GPT values, the former to 40 U/l or more with a maximum about 4 hours after the onset of symptoms, the latter to lower values with maximum after 36 hours or more and/or two HBD values exceeding 75% of the corresponding LDH values above 400 U/l with a maximum about 60 hours after the onset of symptoms, or a combination of one GOT-GPT value and one HBD-LDH-combination, elevated as stated above. (c) Findings at autopsy of myocardial necrosis of an age corresponding to the onset of symptoms.

The age and sex distributions in the two groups studied are shown in Table 1.

The following program was used for analysis of FFA. A catheter was inserted into an antecubital vein. Blood samples were taken in heparinized syringes within 15 min



Fig. 1. Concentrations of individual FFA (mean \pm S.E.M.) on admission in patients with AMI (\square) and controls (\square). The fatty acids are denoted by chain length, number of double bonds.

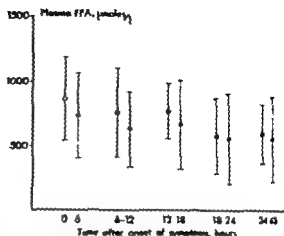


Fig. 2. Total plasma FFA concentrations (mean \pm S.E.M.) in relation to the onset of symptoms in patients with AMI (\circ) and controls (\bullet).

after admission, before the meals at 8 a.m., 11 a.m. and 4 p.m. and 11 p.m. The syringes were immediately placed in ice-water. Plasma was separated and extracted 15 min after sampling. Individual FFA were determined by gas chromatography using heptadecanoic acid as internal standard (3). The error of this method expressed as the coefficient of variation is $\pm 3\%$ for the determination of total plasma FFA. All these blood samples are also used for serum enzyme determinations. Neither heparin nor glucose was administered to any of the subjects.

RESULTS

The total plasma FFA concentration on admission was 830 ± 367 μ moles/l (mean \pm S.D.) in the infarction group and 815 ± 448 μ moles/l in the control group. There were no significant differences in the concentrations of the individual FFA between the two groups (Fig. 1).

There was no significant variation in FFA levels between the samples obtained at 8 and 11 a.m. and 4 and 11 p.m. When the samples were arranged according to the time between the onset of symptoms and the sampling there was a tendency for the FFA values to decrease slightly during the first 48 hours (Fig. 2). This decrease was somewhat more pronounced in the patients with AMI but analysis of variance revealed no statistically significant changes in either group. The intrasubject variation in plasma FFA during the first 24 hours after admission was considerable, the mean difference between the highest and lowest value observed in each individual being 417 ± 143 μ moles/l (mean \pm S.D.).

The infarction group was subdivided according to certain criteria to find out whether the infarctions were associated with high FFA levels on admission. The results are shown in Table II. In the patients who had large infarctions, as estimated from the maximum S-GOT values, there was a tendency towards higher plasma FFA, but the difference was not statistically significant. Left ventricular failure (diagnosed by the presence of pulmonary rales and/or typical X ray finding) was not associated with high FFA levels in the patients with AMI or in the control group. The group of arrhythmias in Table II includes all patients with any kind of disorder of rhythm or conduction during the first 24 hours, with the exception of ventricular and supraventricular extrasystoles. These were excluded since they occurred in all patients except two. The plasma FFA concentrations on admission and during the first 24 hours did not differ between this group of patients with arrhythmias and that without. The degree of pain was estimated from the need for analgesics during the first day and had no significant effect on plasma FFA. However the patients who had non-specific symptoms such as nausea, vomiting or sweating on admission had elevated plasma FFA. This elevation was most pronounced for the two main saturated fatty acids, palmitic and stearic acid, which were both present in higher percentages of the FFA fraction in the group with increased total plasma FFA (Table II).

The time relationship between the development of arrhythmias and changes in plasma FFA was investigated by selecting the samples closest in time to the occurrence of a change in cardiac rhythm or conduction. The material was too small to allow any statistical analysis for the different types of arrhythmias. However the occurrence of auricular fibrillation, atrioventricular blocks and bundle branch blocks was not consistently associated with FFA levels that deviated from the average values for the whole infarction group. On the other hand, in the three patients who developed primary ventricular tachycardia (VT), the last FFA sample obtained prior to the occurrence of the arrhythmia generally showed a remarkably low value. The plasma FFA levels in these three patients are presented in Fig. 3. Patient 1 had plasma FFA of 354 $\mu\text{moles/l}$ on admission and developed VT 45 min later. Pa-

Table II Plasma FFA in patients with AMI

	No. of pts.	Mean \pm S.E. ($\mu\text{moles/l}$)
S-GOT max. (mU/ml)		
> 150	6	1032 \pm 127
< 150	15	773 \pm 90
Arrhythmias	13	868 \pm 115
No arrhythmias	10	754 \pm 99
Need of analgesics		
> 2 times/24 h	8	936 \pm 143
< 2 times/24 h	15	756 \pm 91
Signs of heart failure		
Present	8	786 \pm 132
Not present	14	797 \pm 100
Non-specific symptoms ^a		
Present	9	1014 \pm 142
Not present	11	673 \pm 104

^a Nausea, vomiting and/or sweating.

tient 2 had seven bouts of VT and five of these occurred while he had low FFA levels (311 and 196 $\mu\text{moles/l}$). Patient 3 also had low plasma FFA on admission (234 $\mu\text{moles/l}$). Three hours later he had several attacks of VT during one hour and on two occasions ventricular fibrillation that was successfully treated with defibrillation. Due to the critical condition of the patient no FFA sample was obtained during this time. After these episodes the plasma FFA rose to a normal level of 704 $\mu\text{moles/l}$. Patient 3 died shortly afterwards in a new tachyarrhythmic attack. In none of these three patients were there any signs of pulmonary oedema or shock.

DISCUSSION

The plasma FFA levels observed in both patient groups on admission represented an increase of about 50% above the mean basal level in healthy subjects (3). This increase was not specifically related to the myocardial infarction, since the FFA levels were equally high in the patients who were admitted according to the same criteria as those with myocardial infarction but in whom the diagnosis was later discarded. Analysis of the individual FFA showed that there was a comparatively high percentage of oleic acid in both groups. This is what one would expect at these high FFA levels, since the percentage of oleic acid increases with total FFA concentration (3).

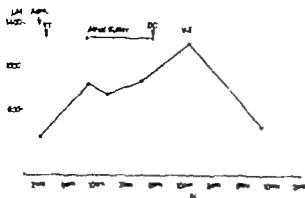


Fig. 3a. Patient 1



Fig. 3b. Patient 2

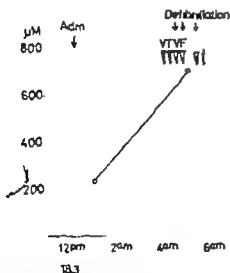


Fig. 3c. Patient 3

Fig. 3 Total plasma FFA concentrations in the three patients with AMI who developed VT

The plasma FFA levels reported in this study are somewhat lower than those observed by earlier investigators (2, 8, 10). This difference may be of methodological origin since the more specific gas

Table III Percentage composition of plasma FFA in myocardial infarction patients with and without arrhythmia-specific symptoms (nausea, vomiting and/or sweating)

Fatty acid ^a	Percentage of FFA	
	Without symptoms	With symptoms
12:0	1.4	1.3
14:0	3.0	3.0
16:0	25.8	27.3
16:1	4.6	3.9
18:0	10.5	11.2
18:1	37.0	37.8
18:2	13.8	11.5
18:3	1.3	1.2
20:1	0.8	1.0
20:3	0.3	0.4
20:4	1.4	1.3
FFA	673	1014 µmol/l

^a Denoted by chain length number of double bonds.

chromatographic method used in the present study is likely to give lower values than those obtained by titration. Another factor influencing the results may be the time delay between blood sampling and extraction of the FFA, which in the present investigation was limited in one hour. Storage of serum, even if frozen, is known to cause a considerable and rapid increase in FFA levels (1).

In a recent study of the levels of individual plasma FFA about one year after a myocardial infarction we observed changes in the concentrations of the polyunsaturated FFA when compared to subjects without evidence of atherosclerotic diseases (4). The patients who had a myocardial infarction showed a lower concentration of arachidonic and octadecatrienoic acid and a higher concentration of eicosatrienoic acid. These changes were not discernible in the present study probably because they were masked by the many factors influencing the plasma FFA level in the acute situation.

Inspection of the data in Table III reveals a general tendency for the FFA levels to be higher in the more severely ill patients. The size of the infarction, the presence of pain and the development of arrhythmias were all factors associated with higher FFA concentrations, although the differences were not statistically significant. The elevated plasma FFA concentration in patients with myocardial infarction thus seems to reflect

the metabolic disturbance caused by the acute Ebcem. This interpretation is in agreement with the observation by Gupta et al. (2) of a correlation between the plasma concentrations of FFA and adrenaline in AMI. It is therefore understandable why some investigators have found a relation between high FFA levels and arrhythmias

while others have not. However the interpretation of this relation proposed by Kurien et al. (7, 8), that the circulating FFA themselves are arrhythmogenic, seems doubtful. These investigators observed a relation between the highest FFA concentration found during the first 48 hours after admission and the development of arrhythmias during the stay in hospital without regard to the time delay between the FFA peak value and the change in cardiac rhythm. The plasma pool of FFA is turning over very rapidly and changes in FFA concentration may occur within minutes. In the present study we observed highly variable FFA levels during the first day in spite of the fact that the sampling was timed so as to minimize the influence of meals. The mean difference between the highest and lowest FFA values during the first 24 hours was about 50% of the mean level on admission. To ascertain a direct effect of plasma FFA on the development of arrhythmias, it is therefore necessary to obtain blood samples as close as possible in time to the change in cardiac rhythm. Since the treatment of these complications involves measures that may influence the plasma FFA level, one has to sample intermittently during the period of study. When this was done we did not observe any relation between the occurrence of auricular fibrillation, atrioventricular blocks or bundle branch blocks and high FFA concentrations in the closest preceding sample. On the contrary there was a tendency for the patients who developedentricular tachycardia to have exceptionally low FFA levels before the occurrence of the arrhythmia. However this observation was limited to three patients and more information is required to substantiate this relation.

Significantly higher FFA levels were observed on admission in the group of infarction patients who had non-specific symptoms. Analysis of the individual FFA revealed that this increase was most pronounced for the saturated acids. This is a surprising finding in view of the fact that an increase in plasma FFA is normally associated

with a change in the fatty acid composition towards a greater similarity to that of adipose tissue triglycerides, i.e. lower percentages of the saturated stearic and palmitic acids and a higher percentage of oleic acid (3). The finding of increased percentages of stearic and palmitic acids in this group with high plasma FFA may indicate that this elevation is not solely caused by an augmented release of FFA from adipose tissue, but that a decreased peripheral utilization of FFA could also contribute.

ACKNOWLEDGEMENTS

This work is supported by grants from the Swedish Medical Research Council (19X-722 and 19X-3401) and from the Wenner Foundation.

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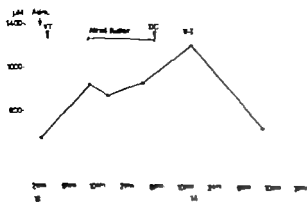


Fig. 3 a. Patient 1

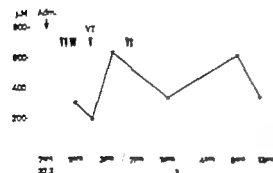


Fig. 3 b. Patient 2.

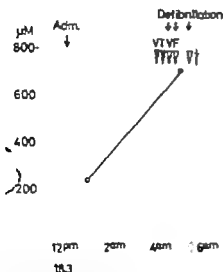


Fig. 3 c. Patient 3.

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A CASE OF MASSIVE HYPERTRIGLYCERIDAEMIA AND IMPAIRED FATTY ACID INCORPORATION INTO ADIPOSE TISSUE GLYCERIDES (FIAT), BOTH CORRECTED BY NICOTINIC ACID

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Abstract. A case of massive chylomicronaemia with fasting plasma triglyceride levels around 100 mmol/l and normal postheparin lipoprotein lipase activity (LLA) has been studied. To investigate other possible mechanisms for the hypertriglyceridaemia than reduced LLA, we have attempted to evaluate the capacity of adipose tissue to assimilate fatty acids split off from plasma triglycerides by LLA by studying the incorporation rate of fatty acids into adipose tissue (FIAT). Adipose tissue incubated *in vitro* showed a lowered FIAT. Also the incorporation of sodium glucose into glyceride glycerol (GLIAT) was low compared to control subjects. After two months' treatment with nicotinic acid, when plasma triglyceride levels had fallen to nearly normal levels, FIAT and GLIAT had increased about 3-6-fold and were then in the range of the control subjects. The role of the capacity of adipose tissue for assimilation of fatty acids split off from plasma triglycerides in regulating uptake of triglyceride fatty acids from the blood is discussed.

There is increasing evidence indicating a close association between hypertriglyceridaemia and ischaemic heart disease (IHD) (1-3, 6, 7, 9, 14), and thus elucidation of the mechanisms causing hypertriglyceridaemia is important. The most pronounced hypertriglyceridaemia is seen in cases with massive chylomicronaemia. A deficiency in lipoprotein lipase activity (LLA) in blood after heparin administration has been shown in some patients with massive chylomicronaemia (14, 15). This has been interpreted as an indication of low tissue content of LLA, which could be a cause of the hypertriglyceridaemia as LLA is believed to split off fatty acids from plasma triglycerides as first event in the removal of plasma triglycerides from blood. Also in some more moderate cases of hypertriglyceridaemia low levels of postheparin LLA have been found (5, 14). Causes

of abnormal chylomicronaemia, other than low LLA, have not been recorded.

We recently reported a case of massive chylomicronaemia and elevated, very low density lipoprotein levels but with normal postheparin LLA (10). Lipheparin rapidly cleared the abnormally prolonged alimentary lipaemia of this patient, with a concomitant rise in plasma free fatty acid (FFA) levels. These findings indicated that the massive hypertriglyceridaemia was caused by mechanisms other than lack of LLA. Our interest in this patient has thus been focused on the chain of events involved in the assimilation by tissues of the triglyceride fatty acids after being split off by LLA. We report here an impaired fatty acid incorporation into adipose tissue glycerides (FIAT) when the patient was grossly lipaemic. This low FIAT was corrected by nicotinic acid at the same time as the plasma triglycerides returned to normal.

CASE REPORT

The patient is a 50-year-old man, described in detail elsewhere (10). His lipaemic fasting plasma, which contained huge amounts of chylomicrons, was discovered accidentally in 1965. The 1-g glucose tolerance was normal, but from 1966 it was slightly impaired. In 1972 the patient was admitted for investigation when his fasting blood glucose varied between 100 and 120 mg/100 ml and the amount of sugar in the urine between 0 and 5 g/4 hours. An 1-g glucose tolerance (0.5 g/kg) performed in March 1972 showed a 1-h value around 0.4% (normal value < 0.110%/min). Measurements of immunoreactive insulin (Phadbas insulin test, Pharmacia, Uppsala, Sweden) at 0, 4, 6, 8 and 60 min gave the following values: 33, 13, 4, 1 and 30 μ U/ml. For men of this age group the level is normally increases from around 10 to about 70 μ U/ml at 4 min (16).

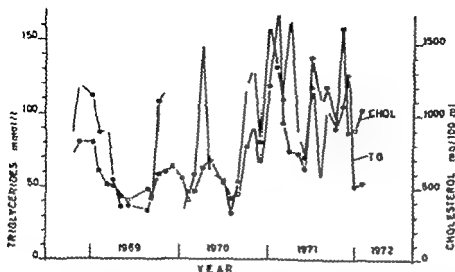


Fig. 1 Concentration of triglycerides and cholesterol in fasting plasma during 1966-72.

The patient's mother had adult onset diabetes with plasma triglyceride levels between 2 and 3 mmol/l. One sister had normal fasting plasma lipid levels. From 1966 the patient complained of occasional pain and paresthesias in the extremities. There have never been any objective neurological signs. From 1971 he had increasing trouble with pains and weakness of his right arm. He was operated on in 1967 for a cancer in situ of the glass penis and has had no recurrence. Otherwise he has been in good health and is clinically normal. Laboratory studies are likewise normal. In particular the ECG at rest, during and after exercise has been normal and there have been no visible or palpable arrhythmias. Since 1967 he has been working as a mechanic. A distinct nerve has sent occasional venous fasting blood samples for plasma lipid analyses.

The patient was hospitalized in March-June 1977 in the Metabolic Ward of the Department of Geriatrics. He was given 400 calories/day the diet containing 40% and 20% calories from carbohydrate, fat and protein, respectively. The carbohydrates were mostly from starch.

Fatty acid composition was 49% saturated, 35% monounsaturated and 16% polyunsaturated fatty acids as determined by gas liquid chromatography.

METHODS

Fatty acid and glucose incorporation into adipose tissue *in vivo*

Abdominal adipose tissue was obtained from the patient in the morning after overnight fast. The last dose of nicotinic acid had been given 14 hours previously to the second biopsy. Subcutaneous adipose tissue was removed under local anaesthesia (1% Xylocain® Astra) using an open biopsy method. Xylocain does not affect the rate of incorporation of ^3H -fatty acids (FIAT) or ^3H -glucose (GLIAT) as determined by permeants in which adipose tissue obtained during general anaesthesia was incubated *in vitro* with 1% Xylocain®. Adipose tissue from the same site was obtained from control subjects who are operated on in the morning after overnight fast under general anaesthesia (Halothane N_2O O_2) for various non-

malignant, non-across disorders (gall bladder disease, 9; arterial disease, 1; hernia, 1; kidney stones, 1; ulcerative colitis, 1). Some of the patients had received i.v. infusion of saline before the biopsy and some i.v. glucose. The maximum amount of glucose was about 6.00 g/kg b.w./min for 30 min or less. The amount of glucose infused did not correlate with FIAT or GLIAT. The tissue is taken immediately after the skin incision. All tissues were treated in the same way and were immediately placed into a large volume of Krebs-Ringer bicarbonate buffer, pH 7.4 containing 2% human albumin (Kabi, Stockholm, Sweden) and 0.1% glucose. They are kept at room temperature for about one hour and then separated from viable connective tissue, cut into 50 mg pieces, and 4 pieces in each flask were incubated at 37°C in 4 ml of the above described buffer containing 0.5 μCi ^3H -glucose/ml (New England Nuclear, Boston, Mass, USA, specific activity 4.4 mCi/mmol) and 0.025 μCi ^3H -palmitate/ml (New England Nuclear, 220 mCi/mmol). The ^3H -palmitic acid was purified by thin layer chromatography (TLC) on silica gel G and then converted to albumin as the sodium salt. For each tissue about 5 incubation flasks were used and the mean value is calculated. After incubation, 2 hours unless otherwise specified, the tissues were rapidly rinsed three times in albumin buffer and extracted in an all-glass homogenizer with 8 ml Dole extraction mixture (13), 3 ml 0.05% KOH being then added. After shaking an aliquot of the heptane phase was washed twice with Dole mixture containing KOH. By this alkaline extraction procedure FFAs are removed. Studies with added amounts of labelled palmitate and triglycerides showed recovery in heptane of less than 0.5 and 94-101% respectively. DL- and monoglycerides were recovered to an extent of 94% and 48% respectively. In control experiments 10 tissues incubated as described above the lipids were extracted with chloroform washed and separated on TLC. The incorporation of label was greater in triglycerides than in diglycerides than in FFA than in monoglycerides and phospholipids, the latter accounting for about 10% of the total incorporation. Similarity around 1% of total glyceride activity is recovered

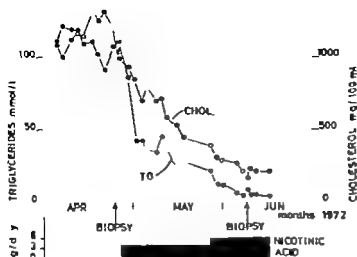


Fig. 2. Effect of treatment with nicotinic acid on fasting levels of plasma triglycerides and cholesterol.

in the monoglycerides when rat fat is incubated with labelled glucose (2) or labelled fatty acids (11).

The heptane extract containing adipose tissue glycerides was divided into two parts, one for counting total glyceride radioactivity and the other evaporated and then hydrolyzed by addition of 4 ml alkaline ethanol (20% KOH in 50% absolute ethanol). The hydrolysis was performed at 60°C for about 20 hours. Then 0.8 ml 12 N HCl was added for acidification to pH 1 and the fatty acids are extracted twice with 4 ml light petroleum ether. The recovery of fatty acids in petroleum ether was 93%. The two lipid extracts containing adipose tissue glycerides and the glyceride fatty acids, respectively were transferred to plastic vials (Packard), evaporated and then 10 ml of solution containing 5 g FPO and 0.3 g POPOP/ml toluene was added. The radioactivity was counted in Packard liquid scintillation counter model 3375 (Packard Instrument Company Inc., USA). The channel settings were so selected that ^3H - and ^{14}C -activity was recovered to an extent of 41 and 9%, in the first channel counting ^3H and of 3 and 84% in the second channel counting ^{14}C . In general about 500 or more cpm were present in the samples which were counted twice for 5 min. External standards are used for quench correction and the amounts of ^3H and ^{14}C in the samples were calculated as described elsewhere (12). The incorporation rate, expressed as nmol, is given here as the radioactivity in the glyceride fatty acids (^3H) and the glyceride glycerol (^{14}C) (obtained as total ^{14}C -activity minus fatty acid ^{14}C -activity) divided by the initial medium specific activity for fatty acids and glucose, respectively. The glyceride fatty acids contained insignificant amounts of ^{14}C -activity. Initial FFA concentration in the incubation medium was 630 nmol/dl and did not change significantly during the incubations. The medium FFA composition was determined by gas liquid chromatography in Pye Unicam Chromatograph 104 on 4% EGSS-X column operated isothermally at 190°C. Palmitic acid, total saturated, monounsaturated and polyunsaturated, made up 24, 34, 43 and 13% respectively.

Other methods

Cholesterol (4) and triglycerides (17) in plasma were determined by semiautomatic methods by the Auto-Analyzer technique. FFA in the medium as estimated by the Dole method (13) after one wash with sulphuric acid.

RESULTS

The variation of the fasting levels of cholesterol and triglycerides in the plasma of the patient since 1968 is shown in Fig. 1. As the normal triglyceride level is about 1 mmol/l, with an upper limit for 95% of the population of about 2 mmol/l, our patient had about 50–100 times the normal level, ranging from 30 to 160 mmol/l. When in the Metabolic Ward, however his triglycerides remained fairly constant (Fig. 2).

Treatment with nicotinic acid was started with

Table 1. Fasting blood glucose (mg/100 ml) and body weight (kg) before and during treatment with nicotinic acid

= number of days when observations were made

	Before	During
Blood glucose		
Mean	103	106
Range	82–128	75–150
	20	40
Body weight		
Mean	69.0	69.7
Range	67.7–70.8	68.7–70.9
	24	38

Table II *Fasting plasma lipids, weight/height (W/H) index and incorporation rate into adipose tissue glycerides in vitro (mean \pm S.E.M., range within parentheses)*

	Normolipidaemic subjects (n=6)	Hyperlipidaemic subjects (n=7)	The patient	
			Before treatment	With nicotinic acid
Age (yr)	47.2 \pm 7.4	46.7 \pm 5.4	30	30
W/H Index ^a	0.95 \pm 0.2 (0.88 \pm 1.00)	1.06 \pm 0.1 (0.89 \pm 1.15)	1.08	1.08
Cholesterol (mg/100 ml)	211 \pm 5	234 \pm 31	1 248	163
Triglycerides (mmol/l)	1.31 \pm 0.07	2.95 \pm 0.58	102	9.06
³ H FA incorporation (l mol/31/g/b)	130 \pm 30 (70-290)	140 \pm 30 (30-240)	30	80
¹⁴ C-glucose incorporation (nmol/g/b)	310 \pm 70 (180-630)	170 \pm 20 (100-240)	30	290

^aWeight (kg)/Height (cm)-100.

1 g \times 4 daily then increased to 1.5 g \times 4 and has since been maintained. Plasma triglycerides slowly fell to normal or near normal levels (Fig. 2). Blood glucose and body weight in hospital before and during treatment with nicotinic acid did not differ (Table I). Since discharge in June 1972 monthly urine tests for sugar and ketone bodies have been negative. The plasma levels of triglycerides and cholesterol have been about 5 mmol/l and 300 mg/100 ml. After about 2 months of treatment the patient no longer complained of symptoms in his right arm.

Both FIAT and GLIAT were considerably lower in the patient than in any of the controls (Table II), who were divided into one group with normal and one with elevated plasma concentration of triglycerides (determined several weeks after operation). There is a tendency for the group with elevated triglycerides to have lower incorporation rates than the normal group particularly for glucose.

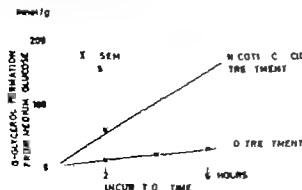


Fig. 3a.

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The patient's FIAT and GLIAT were increased 3-6-fold after 2 months treatment with nicotinic acid (Table II Fig. 3) and were now in the range of the controls. The rate of incorporation before and during treatment with nicotinic acid was linear with time as illustrated in Fig. 3. The plasma triglyceride levels at the times of the two biopsies were 102 and 9 mmol/l (Fig. 2).

Fig. 4 shows the relation between the calculated rates of incorporation of fatty acids and glucose. There was a positive relationship and in all cases except one, the rate for glucose was greater than for fatty acids, the latter being expressed as nmol/3 assuming formation of a complete triglyceride molecule.

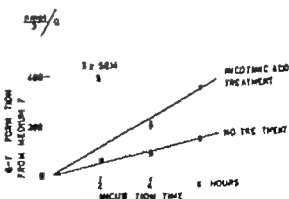


Fig. 3b.

Fig. 3. Rate of incorporation *in vitro* of glucose and glyceride glycerol (GLIAT) (a) and of fatty acids into glyceride-fatty acids (FIAT) (b) in adipose tissue before and during treatment with nicotinic acid. At each interval 5 incubation flasks were used.

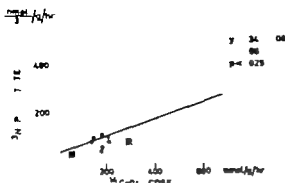


Fig. 4 Relationship between the incorporation rates for fatty acids (FIAT) and for glucose (GLIAT) in adipose tissue of control subjects (○) and the patient (■) before and during treatment with nicotinic acid.

DISCUSSION

The massive chylomicronaemia in our patient is not explained by lack of postheparin LLA (10) as discussed in the Introduction. Since the fasting chylomicronaemia and the delayed alimentary lipaemia is probably caused by an impaired removal of plasma triglycerides, mechanisms other than deficiency of postheparin LLA must be operative. The processes involved in the removal of triglycerides from blood are schematically depicted in Fig. 5. It is evident that a lack of

LLA may slow down the uptake of triglyceride fatty acids. However it is also evident that impairment of the incorporation of fatty acids (FIAT) after the hydrolysis of plasma triglycerides by LLA might cause retardation of plasma triglyceride uptake into adipose tissue. The considerably reduced FIAT of our patient is compatible with such an impairment. The possibility that this defect caused the hypertriglyceridaemia is strengthened by the normalization of FIAT during treatment with nicotinic acid when the hypertriglyceridaemia had been considerably reduced. It is possible that the mechanism by which both nicotinic acid and nicotinamide (10) normalized plasma triglycerides was in their capacity as precursors of NADH/NAD. The slow onset of the effect of treatment on plasma triglycerides (Fig. 2) as well as the persistence of normal plasma triglyceride levels for months after withdrawal of nicotinic acid or nicotinamide treatment (10) would then suggest a slow build-up as well as a slow breakdown of NADH/NAD in adipose tissue. As illustrated in Fig. 5 NADH/NAD is involved in the formation of glyceride glycerol in the reaction



Proof of this hypothesis has to await measurements of these two intermediates in glycolysis

EXTRA-CELLULAR FAT CELL FLUID

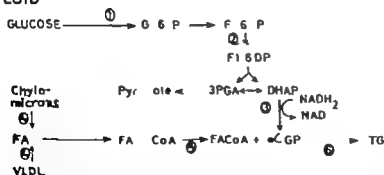


Fig. 5 Processes involved in the removal of triglycerides from blood. Triglycerides of chylomicrons and of very low density lipoproteins (VLDL) are hydrolyzed within the capillaries by LLA and the subsequent emulsification of the fatty acid. Split off involves number of processes before they are incorporated into the tissue glycerides: the fatty acids enter (are transported) into adipose tissue, where

they are activated and then esterified with α -glycerophosphate derived from glucose metabolism to become incorporated into glycerides.

1, membrane transport, hexokinase; 2, phosphofructokinase; 3, glycerophosphate dehydrogenase, NADH_2 , NAD ; 4, lipoprotein lipase; 5, FA activation; 6, triglyceride synthesis.

as well as of NADH/NAD in adipose tissue of the patient.

Nicotinic acid has other effects on adipose tissue which might be linked to our findings of increased incorporation of glucose (GLIAT) and FIAT although they appear to be acute effects and the last dose of nicotinic acid in this study was given 14 hours before biopsy. In vitro addition of nicotinic acid increases glucose uptake (18) in adipose tissue and inhibits fat mobilizing lipolysis (8). After administration to rats in vivo the adipose tissue content of glycogen and a number of glycolytic intermediates, including α -glycerophosphate is increased (20).

The true rate of fatty acid reesterification in adipose tissue cannot be calculated from the present in vitro method of measuring the rate of incorporation of medium fatty acids and glucose because among other things, the specific activity of the immediate precursors to both glyceride glycerol and glyceride fatty acids are not known. Both medium fatty acids and glucose may be diluted by fatty acids from lipolysis and by glucose from glycogenolysis, respectively during their metabolism in the tissue. The ratio between GLIAT and FIAT was fairly similar (Fig. 4) in all cases. This constant ratio suggests that, if dilution of medium glucose and fatty acid occurs before esterification, this was fairly constant in all tissues studied. Furthermore FIAT as well as GLIAT were constant with time over several hours in control tissues as well as in tissue from the patient (Fig. 3), suggesting that any dilution was constant with time. In addition, our data do not reflect the true rate of incorporation because material once incorporated into

cerides may have been metabolized. Also incorporation may have occurred into other pools than the glyceride fraction such as phospholipids and intracellular nonesterified fatty acids. The loss of 50% of the monoglycerides in the extraction procedure is probably of minor importance in this regard, as very little activity was recovered in this fraction (see Methods), unless a specific block in the esterification of monoglycerides to diglycerides may exist in certain cases. However the in vitro technique used was introduced not for estimation of the overall reesterification rate but for obtaining information about the rate of incorporation of fatty acids offered to the tissue from the medium. The idea was that

the incorporation rate of such fatty acids may reflect the capacity of the tissue to assimilate the triglyceride fatty acids split off by LLA (Fig. 5).

The relation between the low incorporation rate in adipose tissue and the patient's mild diabetes is not known. It is evident from Fig. 5 that a defect of glucose metabolism in adipose tissue may cause impaired formation of α -glycerophosphate. The hyperlipidaemia in our patient was, however present in 1965 when glucose tolerance was normal (10); the development of diabetes since 1965 has not worsened the hyperaemia and treatment with nicotinic acid, which improved the adipose tissue defect, did not improve the diabetic condition (Table 1). Also the patient's mother had diabetes without massive hypertriglyceridaemia. Östman has demonstrated a decreased incorporation in vitro of medium fatty acids by adipose tissue from diabetic patients (21). He did not discuss the possible role of the incorporation process in the regulation of the plasma triglyceride levels. However plasma triglyceride concentrations were significantly increased in his diabetic patients with decreased reesterification in adipose tissue. It has also been shown that infusion of glucose and insulin, which might stimulate the formation of α -glycerophosphate in adipose tissue (Fig. 5) lowers the concentration of plasma triglycerides within hours (19).

We do not know if there is a more generalized defect in the tissue handling of fatty acids than a decreased FIAT. However other tissues, and in particular the liver have more abundant glycerokinase activity and may therefore generate α -glycerophosphate from glycerol, thus being independent of the pathway depicted in Fig. 3 for formation of α -glycerophosphate. It is, however of interest in this connection that the skeletal muscle triglycerides increased from 17 to 47 $\mu\text{mol/g}$ during treatment with nicotinic acid (Fröberg, personal communication).

The possible role of the capacity of adipose tissue to incorporate fatty acids into glycerides (FIAT) as a factor in regulating plasma triglyceride levels when LLA is normal has not been investigated in human hyperlipidaemia. From the theoretical point of view (Fig. 5) it is quite possible that reduction of this capacity will decrease the assimilation of fatty acids by the

tissue and if severe enough, may significantly impair the uptake of triglycerides into the tissue and thus their removal from the blood. In addition the findings in our patient and the low values for incorporation seen in some of the hyperlipidaemic subjects in Table II suggest that studies on FIAT and GLIAT in hypertriglyceridaemia could be worth-while.

ACKNOWLEDGEMENTS

Supported by grants from the Swedish Medical Research Council (198-204) and the Nordic Insulin Fund.

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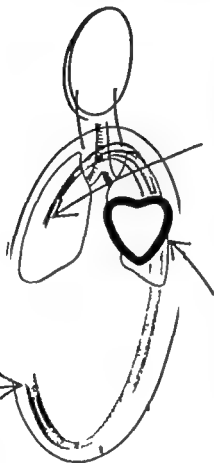
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LYSOLECITHIN AS A FACTOR INFLUENCING ERYTHROCYTE SEDIMENTATION RATE

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Abstract. In an experimental study increasing amounts of lysolecithin has been added to aliquots of plasma from 14 patients with a high erythrocyte sedimentation rate (ESR) and the final concentration of lysolecithin in the samples has been determined by means of thin layer chromatography and subsequent phosphorus determination as well as determination of the ESR. It is found that the ESR remained high in the different plasma samples until critical level of lysolecithin was reached, hereafter the ESR abruptly dropped to lower values. This change took place within a very narrow concentration range, usually between 4.5 and 5.5 μg lysolecithin P/mol, and at a concentration of 7-8 $\mu\text{g}/\text{ml}$ the ESR was consistently very low. In additional experiments, using decreasing amounts of red cells to the various plasma samples, the ESR had normal tendency to increase inversely to the haematocrit, but in this case no threshold level could be observed. These findings clearly demonstrate that the lysolecithin concentration is of considerable importance for the ESR value. A low lysolecithin content of the plasma means higher ESR value than expected from the amount of protein components, and high lysolecithin concentration means low ESR in spite of an unchanged protein pattern. Normally decrease of the ESR takes place on incubation of plasma samples due to the enzymatic formation of lysolecithin from lecithin. This phenomenon is explained by an increase of the lysolecithin amount above the demonstrated critical level. In a number of pathological conditions this "heat-stabilization" process does not occur and in these cases the lysolecithin concentration is characteristically low and does not rise above the critical level even after incubation. A number of possible explanations of this drastic decrease of the ESR: 1. certain lysolecithin level are discussed in this presentation, but the most likely is that lysolecithin is bound to plasma proteins, e. g. albumin. In this form it is unable to exert its physiological effect on the red cell surface membranes until the saturation limit of the carrier protein is reached and free lysolecithin appears.

Since Fåhræus (9) described the sedimentation of red cells in plasma in 1921 an enormous num-

ber of papers have dealt with factors affecting the erythrocyte sedimentation rate (ESR) and especially the plasma constituents enhancing this phenomenon. Fåhræus stressed in the first place the importance of fibrinogen and globulin fractions in this context. He maintained that in acute febrile or inflammatory states the increased ESR could be referred to a raised plasma fibrinogen concentration ("acute ESR increase") whereas a globulin increase was generally found in morbid conditions of a chronic type ("chronic ESR increase").

In his work Fåhræus was also able to show that incubation at body temperature of a plasma with a high ESR caused a considerable reduction of the ESR compared with the basal value of a non-incubated plasma sample. In collaboration with Bergenhjelm (1) he later discussed possible explanations of this incubation effect and, after comprehensive experimental studies in vitro, they postulated that during the incubation of normal plasma an enzymatic degradation of lecithin takes place, probably by splitting off an unsaturated fatty acid radical with the formation of the strongly surface-active compound lysolecithin. By the action of this substance the surface membranes of the red cells will be affected, the cells being transformed from biconcave discs into spherocytes. Hence the erythrocytes were presumed to lose their ability to aggregate and to form "rouleaux" and, since the ESR is directly proportional to the size of the red cell aggregates, the ESR will be reduced.

Bergenhjelm and Fåhræus put forward the concept of a normally occurring heat-stabilization of an erythrocyte suspension, which consequently

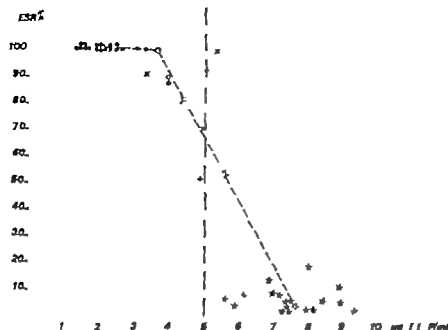


Fig. 1 Correlation between lysolecithin level and ESR in % of the initial value. Lysolecithin added 0 µg (

60 µg (○), 90 µg (□), 120 µg (+) and 180 µg (*). Corresponding mean values: ○ □ + *

was due to the lysolecithin formation produced by a lecithinase present in normal plasma.

Later on it could be demonstrated that the normal heat-stabilization failed to appear under certain circumstances, i.e. in most cases of pernicious anemia (2, 3, 4) and during the acute phase of a viral hepatitis (14). This observation has evoked a renewed interest in recent years, as German authors have claimed that a falling heat-stabilization would make it possible to discriminate ESR increases in malignant conditions

inflammatory disorders. In the former heat-stabilization would not take place (11).

Böttger and Kilbom (8) questioned the accuracy of this assumption and clearly demonstrated that the alleged correlation does not exist. Although a slight difference was found, values for stabilizing and non-stabilizing plasma samples were widely distributed in both disease groups.

In a publication in 1969 Berlin et al. (5) showed that the lysolecithin concentration in normal plasma is remarkably constant in the same individual as well as in different age groups (0.19–0.21 mM lysolecithin phosphorus/l corresponding to 5.9–6.4 µg/ml) with the exception of young men, who have higher values (0.27 mM lysolecithin phosphorus/l = 8.4 µg/ml). After the incubation of plasma at 37°C during 6 hours the

lysolecithin concentration increased by about 100% in all groups.

It is well known that young men have a lower ESR than women of corresponding age. This difference can possibly be explained by the finding that the lysolecithin concentration in young men is about one third higher than in women in spite of the fact that the protein concentration in male plasma is normally somewhat higher which would rather influence the ESR in the opposite direction.

In liver damage the plasma lysolecithin content is considerably below normal level (15). In pregnancy the condition is much the same (15) and sometimes also in contraceptive pill consumers (1). In many cases of pernicious anemia—but by no means in all—and in pronounced iron deficiency anemia the lysolecithin concentration is equally low (Berlin and Vilrot, unpublished observation).

All these conditions usually exhibit a more or less increased ESR. This is certainly due in some part to a change of different protein fraction in cases of liver damage in pregnancy and during contraceptive pill medication. Anemia as such can bring about an increase of the ESR in pernicious anemia and other severe anemia cases. The demonstrated low lysolecithin level, however,

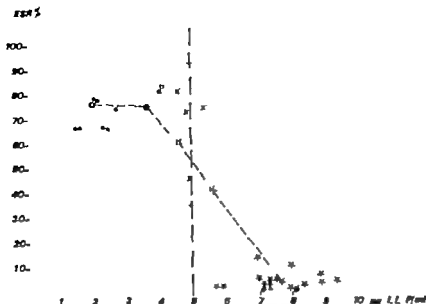


Fig. 2. Correlation between lysolecithin level and ESR in heolite values. Symbols as in Fig. 1.

can also contribute to the raised ESR in cases in which an increased tendency to red cell aggregation will be further supported when a normal lysolecithin effect on their surface membranes is absent.

As an illustrative example may be mentioned the often extremely high ESR in cases of pernicious anemia, which drastically drops to a considerably lower value after specific therapy even before the treatment has influenced the erythrocyte count but when the lysolecithin level has already increased (4).

In order to study these conditions in greater detail and to find out to what extent an increasing plasma lysolecithin concentration will affect the ESR values, and to investigate whether there is any critical concentration limit of lysolecithin below which no appreciable effect on the ESR can be traced, the following experimental study was undertaken.

MATERIAL AND METHODS

Heparinized blood samples were obtained from 18 patients with diseases causing high ESR. The values varied between 50 and 100 mm/hr (Westergren's method). Plasma and cells were immediately separated by centrifugation and kept at 4°C .

Aliquots of 1 ml plasma were diluted with 0.6 ml normal saline solution to which had been added increasing amounts of lysolecithin (0, 40, 80, 120 and 160 µg). Lysolecithin in highly purified form was obtained as an

amorphous powder from Nutritional Biochemicals Corp. Cleveland, Ohio.

The final concentration of lysolecithin in the diluted plasma samples was determined by thin layer chromatography on silica gel and subsequent phosphorus analysis (15).

In the standard experiment 1 ml samples of diluted plasma containing increasing amounts of lysolecithin were mixed with 0.5 ml of the patient's own red cell concentrate and the ESR according to Westergren was read after 1 hour at room temperature.

In an additional experiment plasma samples from one patient were adjusted to three lysolecithin concentrations by addition of saline and lysolecithin. The ESR was then determined after 1 ml diluted plasma had been mixed with decreasing amounts of the patient's own erythrocytes (0.5–0.1 ml of red cell concentrate).

RESULTS

In the standard experiment the ESR was expressed as a percentage of the value obtained with plasma diluted only with saline. The resulting values were plotted against the lysolecithin concentration (expressed as µg lysolecithin phosphorus/ml) in the diluted plasma samples. The mean of the relative ESR values was also determined for every group of samples to which the same amounts of lysolecithin had been added. The results are presented in Fig. 1.

It can be seen that the ESR was not influenced very much by the addition of lysolecithin until a concentration of 4.5–5.5 µg lysolecithin phos-

Table 1 *Lysolecithin levels in diluted plasma after addition of red cells*

Lysolecithin level (μg L L P/ml)	Packed red cells added (ml)				
	0.5	0.4	0.3	0.2	0.1
	Resulting ESR				
20	71	76	80	83	108
4.6	39	31	35	62	76
7.1	5	4	6	8	7

phorus/ml was reached. Further addition of lysolecithin then leads to a pronounced and steep decrease of the ESR. With a concentration of lysolecithin of 7–8 μg phosphorus/ml the reduction of the ESR seemed to be maximal.

In Fig. 2 the absolute value of ESR is plotted for every observation. Similar findings were obtained as when using relative ESR values, although the points are more scattered due to the differing basal levels of the ESR in different patients.

The results of adding varying amounts of red cells to diluted plasma are shown in Table 1. The ESR had a normal tendency to increase inversely with the "hematocrit" and no threshold level could be seen.

DISCUSSION

From the results it is clear that the lysolecithin concentration of a plasma sample is an important factor for the ESR of red cells suspended in this plasma. In all experimental samples the lysolecithin content was the only variable parameter whereas the remaining plasma factors of importance for the ESR value were kept constant throughout. An increase of the lysolecithin content causes a reduction of the ESR. On the contrary when the lysolecithin concentration is lowered, the ESR is increased.

These results also explain how a failing heat stabilization occurs. If the incubation does not bring about an increase of the lysolecithin plasma concentration to levels above 4.5–5.5 μg P/ml, the normal stabilization of the red cell suspension will not appear i.e. no noticeable change of the ESR will occur.

The curves also show that a critical borderline for the lysolecithin concentration can be observed, above which the ESR change takes place rather

abruptly. At lysolecithin concentrations below 4.5 μg P/ml the major part of the ESR values are high, and above 5.5 μg P/ml most values are low.

The biochemical alterations probably develop along a strongly S-shaped curve having a steep part in which even small changes of lysolecithin concentration will alter the ESR value drastically.

Attempts have been made to describe this lysolecithin-dependent change of the ESR in mathematical form. It was possible to construct a theoretical curve that fairly well fitted to the experimental findings, but no additional information was gained by this procedure for a better biological understanding of the phenomenon under discussion.

What, then, may be the cause of the failing heat-stabilization reported to occur in certain pathological conditions, which means that on incubation the lysolecithin does not reach the above mentioned critical level? In the first place it may be due to a decreased content of the substrate, lecithin. However this seems to be less likely as the determination of the individual phospholipids has in no case shown any extremely low lecithin values even after incubation. Rather it may be due to a hampered enzyme activity either a diminished lecithinase activity or a decreased acyl-transferase effect. This lowered activity results in liberation of insufficient amounts of lysolecithin during the standard incubation time of 6 hours. Its concentration level does not exceed the critical limit and the stabilization effect fails to appear.

A further possibility is that an initially very low lysolecithin value does not rise above the crucial concentration limit during the standard incubation time even in the presence of a normal amount of substrate and a normal enzyme activity. This situation may appear in, for instance, cases of pernicious anemia, pregnancy and so forth.

The results of experiments in cases with failing heat-stabilization have demonstrated, however that, after the injection of small amounts of heparin i.v. before the blood sample is drawn, an initially absent stabilization will be normalized (7). This is obviously due to an increased liberation of a lysolecithin-forming enzyme (acyltransferase and/or lecithinase) (6). This favors the

concept that a reduced stabilization effect is caused by a diminished enzyme liberation.

If the aggregation-reducing effect of lysolecithin is dependent on a certain minimum concentration of this compound per erythrocyte, the discussed critical concentration borderline ought to be displaced downwards if the amount of 0.5 ml concentrated red cells per ml diluted plasma is successively diminished. The findings presented in Table I speak against the assumption that a certain number of lysolecithin molecules attached to every erythrocyte is necessary for reducing the aggregation of these cells. Normally on a reduction of the erythrocyte count, a considerable increase of the ESR takes place, which happened also in this experiment. The critical concentration limit is, however not changed, contrary to what might be expected with the distribution of the available lysolecithin over a smaller number of red cells.

A possible alternative explanation is that lysolecithin may interact with another plasma factor. This factor may be the plasma proteins, especially albumin. It is known that most of the lysolecithin in plasma is bound to albumin (13). One explanation of the threshold effect may be that the lysolecithin-binding capacity of the albumin becomes saturated, so that a further addition of even small amounts of lysolecithin causes a break increase of unbound lysolecithin. Perhaps only free lysolecithin can disaggregate the erythrocytes and it may exert this effect even in minute amounts.

It is not known how lysolecithin brings about disaggregation, but possible explanations are a "subhemolytic" action, causing a tendency to spherocytosis (1), or an effect on the electrostatic charges of the red cells.

ACKNOWLEDGEMENT

This investigation has been supported by the Swedish Medical Research Council (grant no. 19X 160-04).

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STUDIES OF LIPOPROTEIN X (LP X) AND BILE ACIDS IN FAMILIAL LCAT DEFICIENCY

Preliminary Report

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An abnormal plasma lipoprotein, designated lipoprotein-X (LP X), has been found in patients with obstructive jaundice (15-17) and has been thought to be typical of this condition. Lithocholic acid is present in relatively high amounts in LP X (14). It has been suggested that the increased concentrations of serum bile acids in obstructive jaundice may be of importance for the formation and accumulation of LP X in plasma (15-17). LP X in obstructive jaundice has been shown to form lamellar structures and discs, probably due to its characteristic lipid composition with high content of esterified cholesterol and lecithin (8).

Many similarities exist between the plasma and erythrocyte membrane lipid composition of obstructive jaundice and familial LCAT deficiency (5, 6, 7, 12). LP X has recently been shown to be present in serum from patients with familial LCAT deficiency (16, 18). Electron microscopical studies have also revealed the presence of lamellar structures in serum and kidneys as well as in "sea-blue histiocytes" in spleen and bone marrow of the LCAT deficient patients (9, 10).

We have investigated the serum bile acid concentration and composition in all the five Norwegian patients with familial LCAT deficiency and also studied the presence of LP X in their serum. Bile composition was studied in two of them. It was found that LP X was present in serum of all patients and that bile acid concentrations were within normal limits both in serum and bile in the two patients studied.

MATERIAL AND METHODS

Serum was obtained from the five Norwegian patients with familial LCAT deficiency (5, 11) after overnight fasting and stored at -20°C before bile acid analysis and at $+4^{\circ}\text{C}$ before LP X investigation. Serum LP X determination was carried out by differential immunoelectrophoretic techniques (13, 15) except that the electrophoretic run was performed with constant current (Seidel, personal communication). The LP X antiserum was obtained from Dr. Seidel, Heidelberg, Germany.

Serum bile acids were determined quantitatively as the

methyl ester acetates using internal standardization as previously described in detail (1). Serum, free and total cholesterol, were determined by gas liquid chromatography (2).

Bile was collected from patient 1 by needle aspiration from gall bladder during operation for kidney transplantation, from patient 5 by duodenal aspiration following L injection of cholecystokinin. All samples were stored at -20°C until analysis. The bile acids were fractionated by thin layer chromatography using different solvent systems (3) and quantitated by enzymatic assay using purified 3- α -hydroxy-steroid-dehydrogenase (4). The conjugates of deoxycholic acid were separated from corresponding conjugates of chenodeoxycholic acid by the methylaldehyde method (3).

RESULTS

LP X was demonstrated in serum from all five Norwegian patients with familial LCAT deficiency. Their serum lipid values (the three of LP X and bile acid studies are documented in Table 1, A) had elevated free cholesterol and markedly reduced concentrations of cholesteryl ester. LCAT activity was not detected in serum of any of them. Total cholesterol was low in the youngest of the female patients (no. 3).

Serum triglycerides were high in all, as were also total phospholipids. Previous investigations have shown that the increase in total serum phospholipids is due to increased concentration of lecithin (11). Elevated concentrations of serum uric acid and acid phosphatases were found in three of the patients.

The serum bile acid studies (Table II) showed that all patients had normal total concentration, $<2 \mu\text{g/ml}$. A peak corresponding to the retention time of lithocholic acid seemed to be the main bile acid constituent in the male patient (no. 5).

None of the patients had any sign of liver or biliary tract disease as judged by the routinely used liver function tests as previously described (4).

The bile composition as studied in gall bladder bile and duodenal bile respectively from two patients was found

Table I. Serum lipid values of five patients with familial LCAT deficiency

Pat. no.	Date (1971)	Total cholesterol (mg/100 ml)	Free cholesterol (mg/100 ml)	Tri-cholesterol glycerides (mg/100 ml)	LP X
1	20.8	182	175	269	+
2	21.9	322	484	770	+
3	19.12	86	79	135	+
4	21.1	171	159	639	+
5	21.1	213	178	620	+

to be normal as to concentration and composition of bile acids and lipids, as seen from Table III. The proportions of taurine and glycine conjugates of cholic, chenodeoxycholic and deoxycholic acids are normal for bile from patient 1. The proportion of conjugates of deoxycholic acid in bile from patient 5 was rather high, though no free bile acids or conjugated lithocholic acid could be demonstrated.

DISCUSSION

Our findings demonstrate the presence of LP X in serum of all patients with familial LCAT deficiency in normal concentrations of serum bile acids and no signs of liver or biliary tract disease. Further our findings from the bile acid studies indicate that no disturbance in bile acid metabolism is present in familial LCAT deficiency. These findings show that factors other than biliary obstruction and serum bile acid accumulation may be responsible for the formation of LP X in serum. The finding of bile acids (particularly lithocholic acid) bound to LP X in patients with liver disease and high serum bile acid levels may probably represent a non-specific binding.

It may be suggested that the plasma LCAT is of importance for the removal of LP X, leading to accumulation of LP X in LCAT deficiency. Increased amounts of plasma free cholesterol and lecithin are common in familial LCAT deficiency and biliary obstructive disease. The possibility therefore also exists that LP X is formed under circumstances where free cholesterol and lecithin are present in plasma in high amounts irrespective of the underlying disease and of the serum bile acid concentration. Further studies are necessary to clarify these problems.

Table II. Serum bile acid concentration ($\mu\text{g/ml}$) in five patients with familial LCAT deficiency

Pat. no.	Date (1971)	Litho-chole	Deoxy-chole	Chenodeoxy-chole	Chole-tal
1	20.8	0.14	ND	0.16	ND
2	21.9	ND	ND	ND	ND
3	19.12	ND	ND	0.11	ND
4	21.1	0.09	0.03	ND	ND
5	21.1	0.93	0.06	0.04	ND

ND = none detected

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Table III. Bile composition in two patients with familial LCAT deficiency

T = taurine conj G = glycine conj C = cholic acid, DC deoxycholic acid, CDC = chenodeoxycholic acid

Pat. no.	Bile acid concentration ($\mu\text{mol/ml}$)	G/T ratio	Phospholipids ($\mu\text{mol/ml}$)	Cholesterol ($\mu\text{mol/ml}$)		
1	23.5	2.3	3.5	1.9		
5	47.0	3.1	4.1	3.0		
Relative composition (%)						
1	81.3		12.1	III		
5	86.9		7.6	5.5		
Individual bile acids ($\mu\text{mol/ml}$)						
	TC	TDC	TCDC	GC	GDC	GCDC
1	4.5	0.9	1.6	8.3	1.2	III
5	3.3	5.0	2.5	8.9	17.0	10.0

ACKNOWLEDGEMENT

This work was supported by a grant from Anders Jahns Research Foundation.

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A CONTRIBUTION TO THE KNOWLEDGE OF THE HYPO- β -LIPOPROTEINEMIA

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Abstract. Low density lipoproteins (LDL) deficiency (hypo- β -lipoproteinemia) was disclosed on routine examination of 25-year-old man. Clinical examination yielded normal findings. Serum was not devoid of LDL. A slightly reduced absorption capacity was found in the vitamin A test and no acanthocytes could be demonstrated in the peripheral blood smear. The dyslipoproteinemia was characterized by low concentration of the β -lipoproteins, high amount to less than one-tenth of normal. In addition there was relative increase in the α -lipoproteins (HDL) content. An essentially similar electrophoretic pattern was found following intake of food. The electrophoretic pattern was normal in the proband's father, brother and two sisters. In the mother (subnormal) content of β -lipoproteins with relative increase in the α -lipoproteins band was found. Analysis of the LDL apoproteins in the proband suggested that the described deficiency could be explained by two defects, i.e. an insufficient biosynthesis of specific LDL apoproteins and insufficient release between the apoprotein and the lipid moiety of the LDL. The possible mode of inheritance of the low density lipoprotein deficiency is discussed and polygenic trait is proposed.

As a genetic disorder apparently unrelated to α -lipoproteinemia, Fredrickson et al. in 1972 (3) introduced as a separate group of dyslipoproteinemias the familial low density lipoprotein (LDL) deficiency (hypo- β -lipoproteinemia), characterized by a plasma concentration of LDL amounting roughly to one-tenth of normal and by the absence of clinical abnormalities. One affected Dutch (1), one French (11) and two American families (6, 8) have been described previously and an autosomal dominant mode of inheritance has been proposed.

In the course of lipoprotein studies on military aviators one individual showing hypo- β -lipoproteinemia was detected. A subsequent more detailed study has been devoted to this proband and

to his relatives. The results of this investigation are presented.

MATERIAL

The proband, white male, aged 25 belongs to group of pilots of the Royal Swedish Air Force undergoing medical examination and electrophoretic screening for lipoprotein phenotypes. His family agreed to be examined on our request.

He has never been hospitalized. Annual health controls have yielded normal results. He claims normal bowel function. His dietary habits revealed an excellent appetite with preference for meat. He admitted to high fat consumption. Bedside examination was non-revealing. BP was 130/80 mmHg. ECG recorded both at rest and during bicycle ergometry showed normal pattern. His physical fitness as above average. On routine laboratory examination serum cholesterol value of 123 mg/100 ml was found.

The proband's mother, aged 60, is children's nurse. Apart from being operated upon for an ovarian cyst and appendicitis she has always been in good health. Her dietary habits are ordinary. Clinical examination revealed slender body build. She looked young for her age. Physical status and ECG are normal.

The proband's father, aged 61, is pensioned. He has earlier been cholecystectomized. A few years previously the diagnosis of hypertensive cardiovascular disease had been made. He has periodically taken insulin. Dietary habits were non-revealing; he consumed alcohol. Clinical examination revealed somewhat obese man. His physical status was normal and BP 160/100 mmHg. ECG showed left anterior bundle block and negative T wave corresponding to the apical area.

The proband's brother, aged 30, is a policeman. Apart from transient complaints of gastric discomfort he has always been well. Clinical examination revealed normally developed body build. His dietary habits were non-revealing. Physical status and ECG were normal.

The brother's wife, aged 32, has always been in good health. They have three children; the youngest, 16-month-old boy was not examined. The other two—a 7-year-old boy and 5-year-old girl—are completely healthy.

Table 1 LDL levels (mg/100 ml) in the proband at three repeated examinations

	Fasting					After food intake				
	Total LDL	β -lipoprot.	Pre- β -lipoprot.	Total LDL cholest.	Triglyceriden	Total LDL	β -lipoprot.	Pre- β -lipoprot.	Total LDL cholest.	Triglyceriden
May	27.5	19.5	8.0	10.9	—	—	—	—	—	—
June	50.0	32.1	17.9	19.1	56.4	75.1	57.3	17.7	30.8	52.5
Oct.	60.0	46.0	14.0	24.3	24.2	170.0 ^b 140.0 ^c	78.9 75.9	91.1 64.1	57.2 49.8	96.6 72.5

6 h after lunch. 4 h after lunch. 7 h after lunch.

The mother's grandfather died of colonic malignancy at the age of 82. He was described as a man looking considerably younger than his age. He was hospitalized during his illness. Serum lipids were not analysed.

Blood samples were taken and immediately analysed after 14–16 hours fasting and again after intake of food containing milk, bread and butter (five slices of bacon, two fried eggs and fried potatoes).

METHODS

Phenotyping the lipoproteins. The procedure used is described in detail elsewhere (10). In brief the total low density lipoprotein level (LDL, VLDL) is estimated by turbidimetry and the sparse lipoprotein electrophoresis of the given serum or plasma is performed. The relative percentages of the β - and pre- β -lipoproteins are derived from the densitometric record of the electropherogram, the sum of β and pre- β being put equal to 100%. The absolute amount of the β and pre- β -lipoproteins in mg/100 ml is calculated from the relative percentage using the absolute amount of the total LDL.

For the turbidimetric estimation of the total LDL, the specific precipitation of LDL + VLDL by dextran sulphate in the presence of calcium ions at pH 9.0 was applied as described by Walton and Scott (13). The specificity of this reaction was checked by electrophoresis and the isolate was reprecipitated with isolated LDL as well (10).

Oil red stained *crude microsomal electropherograms* were produced using both the Beckman cell and densitometer.

Immunological assay. Radial immunodiffusion according to Mancini et al. (7) was performed using both anti- β -lipoprotein serum (Behring) for preparing the plates and β -lipoprotein Parliogen plates (Behring).

Cholesterol and triglycerides. The total LDL cholesterol was calculated from the LDL and VLDL (mg/100 ml) by means of the equation: total LDL cholesterol = 44.9% β -lipoprotein + 22.2% pre- β using the percentages for the cholesterol present in β and pre- β respectively as described by Struss and Wurm (12). Triglycerides were estimated by the method routinely in use at the laboratory (5).

Erythrocytes were examined for the presence of acanthocytes in wet preparations of fresh blood suspended in Dacie's solution (2).

The vitamin A absorption test was performed according to Paterson and Wiggles (9) after administration of 350 000 IU atherophol.

RESULTS

The condition of the proband was disclosed in May 1972. After fasting 14 hours before examination he showed an extremely reduced level of total LDL and particularly of β -lipoprotein (Table I), the concentration of which was only about 5% of the usual value found in normal men, whereas the level of the pre- β -lipoproteins was within normal limits. The β -lipoprotein was never absent. The relative distribution of individual electrophoretic fractions revealed an elevated amount of the α -lipoprotein.

Repeated examinations in June and Oct. 1972 again showed low levels of the β -lipoprotein (Table I and Figs. 1 and 2) corresponding to about one-tenth of the normal, which is characteristic of hypo- β -lipoproteinemia (3). These levels were somewhat higher than in May but the mean value of both amounted to 9% of the normal serum content and the mean value of all three examinations was about 8% of normal. The electrophoresis (table II) showed a repeatedly high percentage of α -lipoprotein.

Acanthocytes could not be detected by microscopic blood examination and the erythrocytes were of normal shape.

By the vitamin A test (Fig. 4), after a dose of 350 000 IU atherophol, a slightly lowered absorption capacity was found.

The effect of food intake was examined twice after previous estimate of the fasting background. It was found that 6–7 hours after the meal the

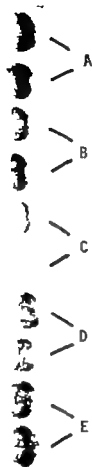


Fig. 1. Electrophoretograms (fasting) of adults of the kindred. (A = father, B = mother, C = proband, D = brother, E = brother's wife.)

β -lipoprotein rose at most to only twice the fasting value (Table I). This was not more than 14–18% of the normal. The plasma contained chyl-

microns (Table II). The relative value for α -lipoprotein (HDL) did not exceed the fasting level, indicating that the newly incorporated fat was not transported in the form of α -lipoprotein.

An attempt was made to obtain preliminary information about the sum of LDL apoproteins in the proband in comparison with three normal individuals. Using the immunodiffusion technique and the β -lipoprotein antiserum Behring, fasting levels amounting to 44–50% of the normal could be detected. There was no significant change after food intake.

Phenotyping of all examined members of the family was performed at the same time, and subsequently the lipoprotein examination was repeated after food intake. Since the values found in the proband have been demonstrated separately in Tables I and II, only the other family members are presented in Tables III and IV whereas Figs. 1 and 3 contain data on the complete kindred. The father and the brother showed normal lipoprotein levels when fasting as well as after food intake. The mother had subnormal lipoprotein values amounting to about 50% of the normal LDL content. Her α -lipoprotein level was in the same high range as that of the proband. After food intake the peak level of her LDL showed a more marked decrease in the 7th hour than occurs normally and the concentration of the VLDL was reduced on the same occasion.

The grandparents were not alive, so that it was impossible to obtain further information on their phenotypes. The proband is single and childless. His sister-in-law showed a slight hyperlipoproteinemia Type IIa. Her son was normal whereas her daughter had a slightly increased LDL content.

Table II. Relative distribution (%) of individual lipoprotein fractions in the proband at three repeated examinations

	Fasting				After food intake			
	Chylomicr	β -lipoprot.	Pre- β -lipoprot.	α -lipoprot.	Chylomicr	β -lipoprot.	Pre- β -lipoprot.	α -lipoprot.
May	0.0	21.3	8.7	70.0	—	—	—	—
June	0.0	23.1	13.8	63.1	8.9 ^a	26.8	8.2	34.1
Oct.	0.0	27.9	8.7	62.6	7.7 ^b	21.2	24.2	46.9
					10.3 ^c	22.4	18.6	48.5

^a 6 h after lunch.

^b 4 h after lunch.

^c 7 h after lunch.

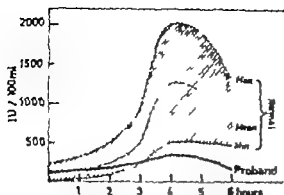


Fig. 4 Vitamin A absorption in the proband after a dose of 150 000 IU xerophthol compared with normal levels.

fact that no acanthocytes were present in the blood, which otherwise are characteristic of abetalipoproteinemia. The vitamin A absorption test yielded a comparatively flat curve the second part being below the minimum according to the criteria of Paterson and Wiggins (9), suggesting delayed fat absorption although, according to the presence of chylomicrons after the food intake, there was no major malabsorption of fat.

The elevated relative level of the α -lipoprotein found in the proband is remarkable. No compensation for the lowered lipid transport in LDL can be presumed, since the level of the α -lipoprotein did not rise after food intake. One possible explanation could be the presence of an abnormal lipoprotein in the α region.

For the explanation of the deficiency found in the proband four factors are of importance. 1) According to the presence of chylomicrons after the food intake there was no evidence of major malabsorption of fat. 2) According to the absence of chylomicrons in the fasting state the lipoprotein lipase was acting properly. 3) LDL + VLDL apoproteins were lowered to about 50% of normal. 4) This amount of apoproteins was higher than that of the whole lipoprotein found. Thus two defects appear to be involved in the deficiency studied, one concerning the biosynthesis of specific apoproteins, the other concerning the linkage between the apoprotein and the lipid moiety of the LDL. It may be argued that not individual specific LDL apoproteins but a sum of antigenic components was evaluated here, resulting in overestimation of the apoproteins. A subsequent more detailed immunological investigation would thus be of value.

As shown by the lipoprotein phenotyping in the kindred, the only similarity was between the proband and his mother. The mother did not fulfill the low LDL level criterion of hypo- β -lipoproteinemia. If she were considered as a case of high LDL level on the Gaussian curve of hypo- β -lipoproteinemia, she would correspond to the scheme postulated by Fredrickson et al. (3) and Levy et al. (6), and an autosomal dominant inheritance could be accepted as regards the proband. On the other hand, if she were classified as a low normal individual, the polygenic model of inheritance could come into question as suggested for familial hypercholesterolemia by Jensen and Blankenhorn (4). Whereas in the dominant inheritance trait a single primary defect and transmission by a single dominant gene is postulated, the polygenic theory claims that the phenotype is determined by two or more additive genes, which may be alleles in one or more loci and which are transmitted through both parents. Such deviant genes with variable additive and similar effects may be insufficient to produce a deviant phenotype in each of the parents, but the progeny may receive enough genes from both of them to produce the deviant phenotype (5). As far as the dominant trait is concerned, a Gaussian distribution with a comparatively high LDL concentration (the mother) appears contradictory to a single primary defect. Furthermore the deficiency in the proband was shown to be probably caused by two factors, insufficient biosynthesis of the apoprotein and defective linkage between the apoprotein and the lipid moiety. At the present stage of knowledge the polygenic trait thus seems to correspond better to the kindred studied since the primary defect of the hypo- β lipoproteinemia has not been discovered so far. When the primary defect is known, without doubt it will be transmitted as a single gene defect.

ACKNOWLEDGEMENTS

The investigation was aided by grants from the Dalcation for Applied Medical Defence Research, Ministry of Defence, Stockholm, and the Scaevada Co. Oskarshamn, Sweden.

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CARBOHYDRATE-STIMULATED FATTY ACID SYNTHESIS DE NOVO
IN HUMAN ADIPOSE TISSUE OF DIFFERENT CELLULAR TYPES

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Abstract. Fatty acid (FA) synthesis *in vitro* by human adipose tissue has been determined as glucose incorporation into FA by slices and as the overall enzyme capacity for incorporation of acetyl units into FA from acetate and citrate by an optimal cytoplasmic assay system. Twelve subjects with normal, hypertrophic, hyperplastic or combined hypertrophic-hyperplastic adipose tissue were examined during basal, weight stable conditions and after 3 and 22 days on high-carbohydrate low fat chemically defined diet (Vivazorb2) administered in amounts resulting in moderately negative energy balances. Incorporations obtained with the cytoplasmic assay were several times higher than those with the whole cell techniques. During carbohydrate feeding FA synthesis increased 3 and 11 times, respectively as measured with the subcellular and the whole cell techniques. However FA synthesis *de novo* was of little quantitative importance in human adipose tissue even during stimulated conditions. Expressed per g adipose tissue triglyceride (TG), there are no differences in FA synthesis between the four cellular groups before or during high-carbohydrate feeding. Expressed per cell, large fat cells demonstrated higher enzyme capacity for FA synthesis than did cells of normal size. The correlation between fat cell size and enzyme capacity for FA synthesis increased during the course of high-carbohydrate feeding. During but not before high-carbohydrate feeding there were several correlations between different whole cell and subcellular activities of adipose tissue and also strong correlations between FA synthesis in adipose tissue and serum TG. The latter correlation was most likely not related to direct dependence between the two factors. Contrary to earlier reports, this investigation indicates the presence of ATP citrate lyase [P.C.4.1.3.8] in human adipose tissue and demonstrates that, *in vitro*, the enzyme capacity for citrate incorporation is at least as large as the capacity for acetate incorporation into FA if optimal conditions are used for both precursors.

In several animals, such as the rat (52) and pig (59), adipose tissue has a large capacity to assimilate and store glucose as triglyceride fatty

acids. The quantitative importance of *de novo* fatty acid (FA) synthesis in human adipose tissue is difficult to evaluate because of discrepancies in available results, as recently reviewed (79). On the one hand some authors conclude that *de novo* FA synthesis is of quantitative importance in human adipose tissue (21-33), on the other hand recent investigations seem to demonstrate that key enzymes in this synthesis are virtually absent (73-74-75-76). Reasonably these deviating results depend not only on differences in feeding states and in the subjects but also on differences in assay techniques. Therefore it was considered advisable to reevaluate this question, utilizing *in vitro* techniques including a recently developed optimal system (79) for subcellular determination of the overall enzyme capacity to incorporate acetyl units into FA.

As described in the present and a following paper (77) experiments have been designed to attempt to evaluate the maximal potential of *de novo* FA synthesis in the total adipose tissue mass. In the present paper volunteers have been subjected to long-term feeding with a high-carbohydrate low-fat diet, a regime known to stimulate *de novo* FA synthesis (2). Furthermore most of the subjects were obese that is, with an increased amount of adipose tissue with chronically insulin-stimulated metabolism (4-48-62, 70).

Obese patients were also selected because, in spite of the abnormally elevated plasma insulin levels (4-48, 62, 70), human obesity is associated with a high incidence of decreased glucose tolerance (19-47-61) and a reduced ability to dispose of ingested glucose (89). One possible ex-

Total fat cell number	Normal 4 $\cdot 10^4$		Large +6.5 $\cdot 10^4$					
Average body fat cell weight, μ	Normal 0.50	Large 0.75	Normal 0.50	Large 0.75				
	1 20 25	4 20 25	7 20 25	10 20 25				
Patient no.	2	5	8	11				
Age years	30 35	30 35	30 35	30 35				
	3 40 45	6 40 45	9 40 45	12 40 4				
Observation no.	12 3 4 5 6	12 3 4 5 6	12 3 4 5 6	12 3 4 5 6				
Group no.	1 Normal	2 Hypertrophic	3 Hyperplastic	4 Combined				
		o o o						
Observation no.	"before 1"		"before 2"		3	4	5	6
DAYS	(-80) (-30) (-10) (0)		(4) (9) (16)		(22)	(23)		
Body composition	— — —							x
Weight	— — —							
4-day records	— — — —							
Nitrogen balance			— — —					
Adipose tissue metabolism			— x —		x			x
Insulin glucose lipoproteins and cholesterol	— — —		x	x	x			x
OGT	— — —			x				x
IGT	— — —				x			
FEEDING	Ad libitum				Carbohydrate			

Fig. 1 Experimental design. The upper part shows the subgroups, the lower part the time course of the experiment. — = examination performed once during the indicated time; — — = continuous or daily events, = examination at indicated time. OGT = oral glucose tolerance test, IGT = 1 glucose tolerance test.

planation of this is a peripheral insulin resistance in different tissues (43 64 69 70 85 89). In this connection the potential of adipose tissue synthesise FA de novo is of interest as one of the major possibilities, theoretically to assimilate and store glucose carbon.

EXPERIMENTAL DESIGN

The present experiment was designed to yield information about fatty acid synthesis in different cellular types of human adipose tissue during steady weight conditions on usual food and during feeding with high-carbohydrate low-fat diet to low- to slightly hypocaloric amounts. (In this paper hypo- low- and hypercaloric feeding refer to the amount of calories required to maintain steady weight.) The experiment was performed on an out-patient basis.

Selection of subjects. Twelve weight-stable, non-diabetic fertile women were selected with respect to three determining factors: (a) total fat cell number (b) average body fat cell size and (c) age. Together with time

(d), then, a, b and c constituted determining factors in the analysis of effect variables. Hence, from

statistical point of view the experimental design is a four-factor balanced block design (Fig. 1) (see Statistical methods). The subjects had an adipose tissue cellularity which was either normal, hypertrophic, hyperplastic or combined hypertrophic-hyperplastic (11, 14) (Fig. 1). In the light of previous investigations (11 12, 14, 16, 17 81) the total fat cell number is arbitrarily considered normal below 4 $\cdot 10^4$ and increased above 6.5 $\cdot 10^4$. Correspondingly the average mean fat cell size was considered normal below 0.50 μ and increased above 0.75 μ . Individual data of the subjects before high-carbohydrate feeding are given in Table I. Table II is statistical analysis of body composition and adipose tissue cellularity in the different groups during carbohydrate feeding. Body weight, body fat and body cell mass were highest in the combined hypertrophic-hyperplastic group followed by the hyperplastic, hypertrophic and normal groups. The choice of groups according to cellularity seemed to bring about relevant experimental design. Thus the average body fat cell weight was almost identical in women with normal and a large total fat cell number (0.64 and 0.63 μ , respectively)

Table 1. Individual data before high-carbohydrate feeding

Group	Pat. no.	Age (y)	Body height (cm)	Weight/ideal weight ^a	Body weight (kg)	Body cell mass (kg)	Extra-cellular water (kg)	Body fat (kg)	Average body fat cell weight (kg)	Total fat cell no. ($\times 10^{-10}$)
Normal	1	20	161	0.99	52.5	19.7	16.5	9.9	0.42	4
	2	33	170	1.01	60.3	22.5	20.7	10.0	0.49	2.0
	3	40	166	1.02	57.2	22.7	18.3	9.4	0.47	2.0
Hypertrophic	4	22	163	1.55	84.0	25.6	18.9	33.0	0.89	3.7
	5	33	165	1.61	87.0	29.3	22.2	28.9	1.09	2.7
	6	40	139	1.55	80.6	26.1	20.6	27.7	0.78	3.6
Hyperplastic	7	21	166	2.00	112.0	30.6	27.1	47.7	0.43	11.1
	8	35	174	2.25	142.5	40.4	48.2	46.3	0.48	9.6
	9	45	168	1.84	106.8	27.8	32.2	39.7	0.47	8.4
Hypertrophic	10	23	158	2.08	120.8	30.6	27.2	56.0	0.83	8.8
hyperplastic	11	38	177	2.15	140.0	41.9	26.8	63.6	0.87	7.3
	12	40	162	2.48	134.0	31.6	32.0	63.9	0.76	8.4

^a relation to length, from Metrop. Life Insur. Co. (cf. 26).

At the same time the numbers of fat cells are similar in women with normal and increased fat cell sizes (5.8×10^9 and 5.4×10^9 fat cells, respectively). There were no differences between the three age groups with respect to body fat or adipose tissue cellularity (Table II).

Schedule of the experiment. The first examination was performed 10–80 days before the start of high-carbohydrate feeding ("before-1") and the second ("before-2") 1–10 days before the start, as shown in Fig. 1. Observations 1, 4, 5 and 6 were done on the 4th, 9th, 16th and 23–23rd days, respectively of high-carbohydrate feeding. Body composition as determined 1–3 months before the experiment. The patients were then weight-stable (Table II bottom section) up to the start of high-carbohydrate feeding. This was achieved simply by asking the patients not to change their food habits. Fluctuations in weight paralleling the menstruation cycle had to be accepted. The experiment was started so that menses occurred between the 14th and 19th days of high-carbohydrate feeding in all women except no. 6 who had her menses on the 6th day. Body composition and adipose tissue cellularity were reexamined on the 23rd day of the experiment.

Adipose tissue metabolism as determined during "before-2" usually 2 days before the start of high-carbohydrate feeding, and on the 4th and 23rd days of the experiment. Plasma insulin, blood glucose and serum lipids are determined during "before-2" and on the 4th, 9th, 16th and 23rd days of the experiment. Oral glucose tolerance tests are performed 10–30 days before and on the 4th and 22nd days of the experiment. Intravenous glucose tolerance tests are performed 10–30 days before and on the 16th day of the experiment.

Nutritional and energy balance. The intake of energy and nutrients during weight-stable conditions are estimated from four 4-day dietary records obtained during the last month before the experiment. The patients were given detailed oral and written instructions by dietitian

how to register and report in common household measures everything eaten during the four 4-day periods, meal for meal. From these records the mean intake of calories, protein, carbohydrate and fat was calculated by means of food composition tables (1) and own food analyses (Table III). There were no statistically significant differences between the subgroups with respect to energy intake or distribution of protein, fat and carbohydrate.

In order to check the dietary records, 24-hour specimens of urine were collected during the last 4-day period and analysed for nitrogen. As the average sum of fecal and dermal nitrogen losses in normals is close to 2 g/day (46, 63, 67, 86), the protein intake can be estimated during steady state conditions from urine nitrogen analyses. The data obtained indicate that the recorded intakes of nitrogen were lower than actual, especially among the obese subjects (Table III). This holds true also for energy (45).

It was the primary intention of the study to give the subjects isocaloric amounts of high-carbohydrate low-fat diet. Taking into account the general credibility of the dietary records and also the discrepancy between recorded nitrogen intake and the intake estimated from urinary analyses the individually prescribed caloric intake during the experiment is adjusted upwards as compared with the results of the 4-day records.

The high-carbohydrate low-fat feeding during the experiment was achieved by means of chemically defined formula diet (Vivasorb® Boloni, Molndal, Sweden). The exact composition was worked out by Wiautz et al. (40, 90). The caloric distribution as: glucose 46.0%, glucose 1.4%, glucose 89.4%, inulin 0.7%, and free amino acids 1.5%. Vivasorb® also contained water-soluble and fat-soluble vitamins, minerals, emulsifier and artificial colouring and flavouring. The individually prescribed amount of Vivasorb® was divided into seven meals between 8 a.m. and 8 p.m. Except for

Table II. Body composition and adipose tissue cellularity in 12 women during high-carbohydrate feeding

Determining factors ^a			Body weight (kg)			Body cell mass (kg)			Extracellular water (kg)		
Fat cell		Age (y.)	Time (day no.)	No. of subj.	Least signif. diff. ^b		Least signif. diff.		Least signif. diff.		
Number	Weight				Means	p	Means	p	Means	p	
Normal	Normal			3	55.9		21.3		18.7		
Normal	Large			3	82.4		26.7		20.6		
Large	Normal			3	118.4	6.6	32.8	4.5	33.8	8.8	
Large	Large			3	129.9		34.3		29.5		
Normal				6	99.1		24.0		19.7		
Large				6	124.1	<0.005	33.6	<0.005	31.7	0.005	
	Normal			6	87.1	<0.005	27.1	<0.01	26.3	n.s.	
	Large			6	106.1		30.5		25.1		
		20-43		4	90.3		26.3		22.6		
		30-39		4	106.5	5.0	32.8	3.5	29.6	6.1	
		40-49		4	93.1		27.3		24.8		
		Before-1	12	98.1			29.1		25.9		
		Before-2	12	98.0							
		4	12	96.7	n.s.			n.s.		n.s.	
		9	12	96.1							
		16	12	95.4							
		23	12	95.5			28.5		25.5		
Grand mean					96.6		28.8		25.7		

In the first horizontal section differences between normal (group 1, -3, of Fig. 1), hypertrophic (group 2, -3), hyperplastic (group 3, -3) and combined hyperplastic-hypertrophic (group 4, -3) patients are analysed with respect to different effect factors (body weight, body cell mass, etc.). In the second section the difference between patients with normal total fat cell number (groups 1+2, -6) and patients with an increased total number of fat cells (groups 3+4, -6) is analysed with respect to different effect factors. In the third section the difference between patients with normal (groups 1+3, -6) and increased (groups 2+4, -6) average body fat cell size is analysed. In the fourth section differences between the three age groups (*n*=4 each group), in the fifth section differences between observations are analysed. Before-1 was performed during weight-stable conditions, usually 1-3 months before the experiment. Before-2 was 1-10 days before the experiment (cf. Fig. 1). Observations at different times were pooled when comparing different groups and all the patients (*n*=12) were pooled when analysing the effect of time.

^a At 5% level according to Turkey. For further explanation, see Statistical methods.

water the patients were not allowed to take anything else mouth.

During the whole experiment urine was collected in 4-hour specimens for analyses of nitrogen. As the intake is well defined by the formula diet, and as the average and variation in fecal and dermal losses are well known from earlier balance studies during metabolic ward conditions (46, 63, 67, 86), the "balance" for nitrogen could be estimated (Table III). A control of the nitrogen "balance" was achieved by estimations of changes in body cell mass, extracellular water and body fat (see Methods). The relatively good agreement between the calculated nitrogen "balance" and changes in body cell mass (-26 g N and -0.6 kg BCM; -24 g N) (58) suggests that the patients had adhered to the prescribed diet. Nevertheless from the changes in body fat it can be estimated that the energy expenditure during the experiment exceeded the intake by some 700 kcal/day. The study was thus performed in a condition of moderately negative energy balance, which also explains the negative nitrogen balances.

METHODS

Body composition

Body cell mass (BCM) was calculated from body potassium as described by Moore et al. (58). Body potassium was determined with whole body counter detecting naturally occurring ⁴⁰K (K2). The counter was calibrated by administering known amounts of ⁴⁰K to volunteers (3). Total body water (TBW) was determined by administration of tritiated water (55). From body weight and height, BCM and TBW extracellular water and body fat could be calculated as described by Berg and Imkinson (7).

Adipose tissue cellularity

The mean fat cell weight of region was determined in needle biopsy specimens by the microscopic method of Sjöström et al. (80).

The average body fat cell weight was calculated as an average of the mean fat cell weights from three regions: 1) The hypogastric region, on a line between

Body fat (kg)			Average body fat cell weight (μ g)			Total fat cell no. (10^{-9})			Mean fat cell weight in biopsies used for metabolic studies (μ g)		
Means	<i>p</i>	Least signif. diff.	Means	<i>p</i>	Least signif. diff.	Means	<i>p</i>	Least signif. diff.	Means	<i>p</i>	Least signif. diff.
9.1			0.45			2.0			0.30		
23.8			0.84			3.3			0.64		
44.8		5.8	0.47		0.11	9.6		2.2	0.46		0.10
79.3			0.81			7.4			0.68		
19.8			0.64			2.8			0.47		
52.0	<0.01		0.63	n.s.		8.5	<0.005		0.57	<0.005	
26.9			0.46			5.8			0.38		
44.0	<0.01		0.82	<0.005		5.4	n.s.		0.66	<0.005	
35.0			0.63			5.8			0.50		
36.9			0.69			5.5	n.s.		0.59		0.08
34.6	n.s.		0.60	n.s.		5.6			0.48		
34.3			0.67			5.7			0.54		
	n.s.			<0.10			n.s.		0.52	n.s.	
34.6			0.61			5.6			0.51		
35.5			0.64			5.6			0.52		

the umbilicus and left superior iliac spine one third from the latter [3]. The femoral region, on the ventral side of the thigh on a line between the patella and the superior iliac spine one third from the patella. 3) The gluteal region, the upper lateral quadrant of the gluteal region.

The total fat cell number of the body was estimated by dividing body fat by the average body fat cell weight.

Blood and serum analyses

Unless otherwise stated, all serum blood samples were taken after an overnight fast on the days shown in Fig. 1. Blood samples were always taken before adipose tissue biopsies. After extraction according to Folch et al. (34), serum triglycerides (TG) were determined as described by Carlson (22) and serum total cholesterol by the method of Cramer and Isaksson (24). Blood glucose was determined by the glucose oxidase method of Keston (43) as modified by Levin and Linde (53). The GlucoB assay kit (Kabi, Stockholm, Sweden) was used. Plasma insulin was determined by the radioimmunochemical method of Hales and Randle (43) using the Phadebas assay kit (Pharmacia, Uppsala, Sweden). In the oral glucose tolerance test patients fasted 800 g glucose in 300 ml water. Glucose and insulin levels were determined before and 30, 60, 90 and 120 min after the glucose intake. In the intravenous glucose tolerance test 25 g glucose was injected over 3-min period and the glucose concentrations were determined before and 20, 25, 30, 40, 50, 60 and 70 min after the injection.

A K_{10} -value was calculated as described by Courat et al. (23).

Serum lipoprotein electrophoreses were performed by Dr A. Gustafson, Gothenburg. An agarose gel was used principally as described by Rapp and Kahlin (65), utilizing a closed, water-chilled chamber described by Lowell (51). Lipoprotein bands are developed by staining in mixture of Oil Red O and Fast Red 7 (Ciba). The serum lipoprotein patterns were typed according to Fredrickson and Lees (36), including the subtypes II A and II B as suggested by Barnwood et al. (5).

Nitrogen analyses of urine were performed at the Institute of Clinical Nutrition, Gothenburg, by means of micro Kjeldahl method using Technicon AutoAnalyzer with digester model 1 (Technicon Controls, INC, Chatham, New York, USA).

Adipose tissue analyses

Biopsy techniques. After intracutaneous anesthesia (1% 2% lidocaine (Xylocain[®] Astra, Södertälje, Sweden) (3 ml along 6 cm long line), an approximately 10 mm deep incision was made with scalpel. About 10 g adipose tissue was rapidly removed with pair of scissors from the deepest adipose tissue layers. In lean subjects adipose tissue close to the skin had to be used, with increasing risk of contamination with lidocaine. The biopsy was placed in Krebs-Ringer bicarbonate buffer (33) (half the prescribed CaCl_2 concentration) at room temperature and processed immediately.

All biopsies were performed in the hypogastric region

Table III Individual data for 4-day records, nitrogen "balances" and changes in body composition in 12 women selected for high-carbohydrate feeding

Group	Subj no.	Differences between nitrogen recorded during the last 4-day record and estimated nitrogen output ^a (g)	Mean daily caloric intake from 4-day records (kcal)	Caloric distribution from 4-day records (%)			Prescribed daily caloric intake during high-carbohydrate feeding (kcal)
				Prot.	Carbo.	Fat	
Normal	1	-4.8	1 150	14	43	40	1 600
	2	+7.9	2 250	12	49	40	2 250
	3 ^a	+0.4	2 400	13	43	44	2 400
Hypertrophic	4 ^a	-14.9	1 350	13	46	41	1 700
	5	-12.1	1 950	11	51	38	2 050
	6	+1.2	2 500	10	44	46	2 750
Hyperplastic	7	—	1 650	17	44	39	1 900
	8	19.1	1 700	10	49	41	2 150
	9 ^a	-38.6	1 200	17	43	40	2 100
Hyperplastic hypertrophic	10	-8.3	2 300	14	34	62	2 600
	11	+1.4	2 400	14	44	42	2 650
	12 ^a	15.7	2 800	12	44	44	2 800
Mean		-6.5	2 000	13	44	43	2 250

^a Incomplete collection of urine during high-carbohydrate feeding was suspected.

^b Developed leucemic reticulocytoblasts (6) 4 months after the experiment.

For technical reasons the second determination of total body water was suspected to be too low. Therefore the decrease in extracellular water and the increase in body fat during high-carbohydrate feeding are uncertain.

Nitrogen output estimated from urine analyses plus 4 g in extraordinary nitrogen losses (44, 63, 67, 86).

Extraordinary nitrogen losses estimated to 23 g (44, 63, 67, 86).

1 a lev 1 midway bet een the umbilicus and on pubis. The first biopsy was taken to the right, the second to the left and the third in the midline between the two former biopsies.

Glucose incorporation into CO₂, glyceral-glycerol and fatty acids determined in whole cell technique. T pieces of adipose tissue, each about 200 mg, were incubated in duplicate in closed, freshly siliconized glass vials containing 5 ml Krebs-Ringer bicarbonate buffer (half the prescribed CaCl_2 concentration) with 4 g% bovine albumin (Fraction V Batch SA 1470 Armour Pharm. Comp. Ltd, Eastbourne, England) and 4.5 mM glucose with 15×10^4 cpm of U- ^{14}C glucose (NEC-042H New England Nuclear, Langen, West Germany). Insulin (mono-component pork insulin, Lot No. MC-S-970-Ac, Novo, Copenhagen, Denmark) to a concentration of 1 000 $\mu\text{U/ml}$ was added, as indicated $\text{O}_2/\text{CO}_2/94.3$ (/) constituted the gas phase and the pH was 7.4. The incubation vessels were furnished with suspended micro-caps of glass containing a piece of filter paper. During the 3 hours' incubation the vessels are shaken at 100 c/sec in metabolic shaker 1370°C.

At the end of the incubation 0.3 ml Hyamine B (Packard, La Grange, Ill., USA) was added to the micro-caps and 0.1 ml 1 M NaHCO_3 and 0.3 ml 1 N H_2SO_4 were added to the incubation medium through covering membrane with a syringe. The vessels were then shaken again for at least 30 min. The micro-caps with

filter paper were taken to vials containing 10 ml toluene with 0.4 g 2,5-diphenyl-oxazole (Packard, La Grange, Ill., USA) and 0.01% p-bis-2(5'-phenyl-oxazolyl)-benzene (Packard) and counted in a Packard tri-carb liquid scintillation counter. Quenching was corrected for by the external standard method in this and the other determinations.

The incubated adipose tissue was rinsed three times in 1 ml normal saline (30°C) and then taken for extraction overnight in 5 ml of a mixture containing 1 M H_2SO_4 , heptane and isopropyl alcohol (10:40:50) as described by Dole (77) and Dole and Melnick (78). After addition of 4 ml heptane and 4 ml ether the resulting heptane phase was washed twice with 4 ml 0.05% sulphuric acid as described by Trout et al. (77). The adipose tissue residue was reextracted once as described above. The two heptane phases are pooled and 1 ml in duplicate was then taken to counting tube. The radioactivity as considered to be present in the triglycerides. The remaining heptane phase was taken to dryness in a pre-weighed Kewmax tube (Kabo, Molecular, Sweden) and the TG residue as determined by weighing. From this the TG content of the incubated adipose tissue was calculated. Ethanol, 10 ml, and 10 M KOH 1 ml, were added to the Kewmax tube and the content was boiled for 60 min with the teflon screw cap on. After cooling and acidification with 1 ml conc. HCl the saponified fatty acids were extracted with 6 ml

Cumulative acetate
"balances" during
11 days of high-carbo-
hydrate feeding* (g)

Changes in body composition during high-carbohydrate feeding (kg)

	Δ Body weight	Δ Body cell mass	Δ Body fat	Δ Extracellular water
- 30	-2.1	+0.2	-1.7	-0.6
- 3	-0.9	-0.1	-0.3	-0.4
+ 10	-1.6	-1.9	-2.0	+2.3
+ 26	-5.5	-1.2	-4.6	+0.3
+ 43	-2.0	-2.5	+1.6	-1.1
- 4	-0.3	+2.1	-3.4	-1.0
26	-4.4	-1.3	-1.7	-1.4
122	-4.5	-1.4	+0.3	-3.4
-137	-2.3	+2.0	+2.8	-7.1
4	-2.8	-0.5	-5.3	-3.0
- 47	+0.4	-1.7	-3.9	-6.0
90	-6.0	-0.1	-2.1	-3.8
26	-2.6	-0.6	-1.7	-0.4

heptane. The radioactivity of 2 ml heptane was counted in duplicate as described above. The incorporation of glucose into glyceride-glycerol was calculated as the difference between incorporations into TG and FA.

Blank values are obtained by taking boiled adipose tissue through all the steps described above.

The incorporation of glucose into the end products was expressed in nmoles per g TG and hour or per 10^6 fat cells and hour.

Fatty acid synthesis determined with subcellular technique. An optical cytoplasmic assay system, described in detail previously (79), was used to determine the capacity of human adipose tissue enzymes to synthesise fatty acids *de novo* from labelled acetate and citrate.

Four tubes containing 30 mM citrate were incubated. They are identical in all respects except that two of these contained $2 \cdot 10^6$ cpm of [$1\text{-}^{14}\text{C}$] acetate (NEC 08-4H, New England Nuclear, Langer, West Germany) and the other two $8 \cdot 10^6$ cpm [$1\text{-}^{14}\text{C}$ 3-C 14] citrate (NEC 140, New England Nuclear). Four other tubes containing 110 mM citrate were incubated in the same way with either the acetate or the citrate labelled.

Acetate incorporation into fatty acids with 30 mM or 110 mM concentration of citrate in the assay system will be referred to as *A 30* and *A 110* respectively. Similarly citrate incorporation in these citrate concentrations will be referred to as *Cit 30* and *Cit 110*. Since the assay conditions were identical for acetate and citrate incorporation with 30 mM concentration of citrate in the assay system, it was possible to add the values for acetate and citrate incorporation, resulting in values of the total incorporation of acetyl units from acetate and citrate. This total incorporation with 30 mM citrate in the assay system will be referred to

as *FA 30*. In the same way the added acetate and citrate incorporations with 110 mM citrate in the assay system will be represented by *FA 110* in order to detect the maximal incorporation of acetyl units in any human adipose tissue tested. It proved necessary (79) to test with both 30 and 110 mM citrate in the assay system. The highest values of *FA 30* and *FA 110* for each individual will be referred to as *FA-max*.

Statistical methods

The observations on the 12 subjects are statistically divided into two classes of variables. 1) Block factors or determining factors. These are: fat cell number, fat cell size, age, time. 2) Effect variables; all other variables (*Y*-variables).

As the design of the experiment is balanced block design, the following relation is assumed to be valid for every *Y*-variable.

$$Y_{ij} = \mu + N + S + A + T + E_{ij}$$

where μ = constant, N = fat cell number group ($N = 1, 2$), S = fat cell size group ($S = 1, 2$), A = age group ($A = 1, 2, 3$), T = time ($T = 1, 2, 3, 4, 5$ and or 6), E_{ij} = random, normally distributed component. For clarification of j , k and i cf. Experimental design.

By common analysis of variance it is hence possible to analyse the difference between the mean values of the *Y*-variables in the different groups (fat cell number groups, fat cell size groups—also the subgroups with normal, hypertrophic, hyperplastic or combined hyperplastic-hypertrophic adipose tissue—and age groups) as well as the changes with time.

The numerical calculations are carried out on an

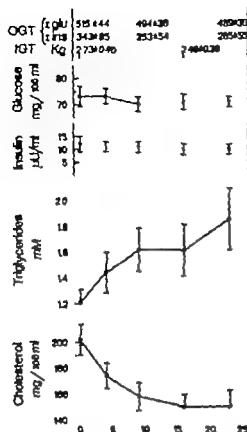


Fig. 2. Effect of carbohydrate feeding on serum lipids, plasma insulin, blood glucose and glucose tolerance (mean \pm S.E.M.).

IBM computer according to the program BMD QTV in Analysis of variance for factorial design (18).

When the patients are divided into only two groups, the significance of the difference between the \bar{Y} -mean values is examined through the corresponding F -value.

When more than two groups are compared and when the corresponding F value in the analysis of variance causes significant differences between \bar{Y} -mean values, so-called "least significant difference" is calculated according to Tukey (see 54) in order to compare any two mean values. If the difference between two means is larger than the least significant difference, these two means are significantly different on at least the 5% level.

The product-moment correlations between different variables were examined by the ordinary t -test (25).

RESULTS

Blood glucose, plasma insulin, glucose tolerance and serum lipids

High-carbohydrate feeding did not significantly change fasting blood sugar, glucose tolerance tests, fasting insulin or sum of insulin values during oral glucose tolerance test (Fig. 2). Serum

cholesterol decreased significantly ($p < 0.005$) during high-carbohydrate feeding, while the increase in serum TG was not fully significant ($p < 0.10$) when evaluated by analysis of variance. If paired comparisons of the serum TG values before and on the 23rd day of high-carbohydrate feeding are analysed with the t -test, the increase is significant ($p < 0.01$).

At the start of the experiment all the subjects had normal serum lipid electrophoresis patterns. However, subject 10 was classified as type II B four months earlier and as type IV only a few weeks before the experiment. This woman immediately shifted to and maintained a type IV during the high-carbohydrate feeding. In subjects 3, 5 and 6 type IV occurred in one or two of the four observations during high-carbohydrate feeding.

Adipose tissue metabolism in vitro

Fat cell size of surgical biopsies. The mean fat cell size of the adipose tissue region where the

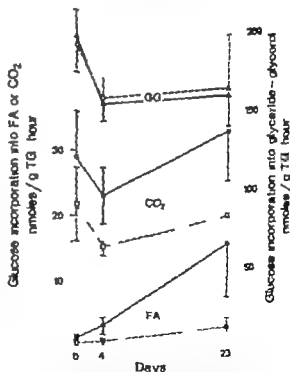


Fig. 3. Effect of carbohydrate feeding on glucose incorporation into glyceride-glycerol (GG), CO_2 and FA by slices (mean \pm S.E.M.). Δ , \square , \circ — basal incorporation; Δ , \square , \circ — 1000 μ U insulin/ml added. For other assay conditions, see Methods. Significant differences, see text.

Table IV Glucose incorporation into FA in adipose tissue slices from 12 women during high-carbohydrate feeding

				Glucose incorporation into FA (nmoles/ μ g fat cells/hour)						
Determining factors ^a				Basal			Insulin-stimulated			
Number	Weight	Age (y)	Time (day no.)	No. of subj.	Means	P	Least signif. diff.	Means	P	Least signif. diff.
Normal	Normal			3	3.7			16.9		
Normal	Large			3	14.1			83.1		
Large	Normal			3	2.6	n.s.		6.8	n.s.	
Large	Large			3	4.5			31.4		
Normal				6	8.9			30.0		
Large				6	3.5	n.s.		19.1	n.s.	
	Normal			6	3.2			11.9		
	Large			6	9.3	<0.10		57.2	<0.10	
		20-25		4	4.0			22.6		
		30-35		4	3.5	n.s.		13.2	n.s.	
		40-45		4	11.1			67.9		
			Before-2	12	2.3			7.0		
			4	12	3.5		9.9	15.0		70.0
			23	12	12.8			81.7		
Grand mean					6.2			34.6		

^aFor explanation of the statistical analyses see Table II and Statistical methods.

surgical biopsies were taken was on the whole somewhat smaller than the average mean fat cell weight used for classification of patients (Table II). However there was a strong correlation between the mean fat cell weight of the surgical biopsies and the average body fat cell weight ($r=0.88$, $P<0.001$, $n=24$).

Whole cell technique. The glucose incorporation into glyceride-glycerol, CO_2 and FA by adipose tissue slices is presented in Fig. 3. High-carbohydrate feeding for 22 days increased the basal glucose incorporation into fatty acids from 0.4 to 2.6 nmoles/ μ g to-tissue ($P<0.01$) and the insulin-stimulated incorporation from 1.4 to 15.4 nmoles/ μ g to-tissue ($P<0.025$). On the 4th day of high-carbohydrate feeding the increases of these incorporations were not yet statistically significant.

In absolute values the insulin stimulation before on the 4th and 23rd days was 0.9 (n.s.), 2.5 ($P<0.05$) and 12.8 ($P<0.10$) Δ nmoles of glucose into FA/ μ g to-tissue, respectively (pairing design *t*-test). There were no fully significant

differences between the absolute values of the insulin stimulations before, on the 4th and 23rd days ($P<0.10$, analysis of variance). The insulin stimulation before, on the 4th and 23rd days was 172, 268 and 472% of basal incorporation, respectively a statistically significant increase with time (23rd day-before $P<0.025$ analysis of variance).

When glucose incorporation into FA was expressed per cell, there was a trend to greater basal and insulin-stimulated incorporation in large than in small fat cells (Table IV).

Glucose incorporation into CO_2 and glyceride-glycerol did not change significantly during the course of high-carbohydrate feeding (Fig. 2). Inulin stimulated the incorporation of glucose into CO ($P<0.001$) but not into glyceride-glycerol.

Subcellular technique Incorporations of acetyl units from acetate and citrate into FA by the cytoplasmic assay system are given in Tables V and VI. Ac 30, Ac 110 Ctr 30, Ctr 110 FA 30 FA 110 and FA-max all increased approximately threefold after 3 days of isocaloric high-carbo-

Blood chemistry

In the present study high-carbohydrate feeding on an average increased serum TG 53% and decreased serum cholesterol 25%. Similar results have been reported previously (9, 10, 33, 37, 50, 90). There was an increasing correlation between serum TG and the subcellular FA synthesis during the course of high-carbohydrate feeding, and a highly significant correlation between changes in serum TG and changes in whole cell FA synthesis. The reasons for these associations can only be speculated on. Kuo et al. (50) suggested that carbohydrate-induced hypertriglyceridemia in man is related to an increased synthesis and release of FA in/from adipose tissue with subsequent flux to the liver where the fatty acids synthesized by adipose tissue are converted to very low density lipoproteins. However this suggestion does not seem to be supported by the data presented by Kuo et al. since the FA release from adipose tissue was much higher than the maximal rate of FA synthesis in this tissue. Recent investigations demonstrate that the total production of TGFA from the liver is in the order of 3 000 μ Eq/hour (29). This secretion of lipoprotein FA from the liver is 10–20 times higher than the extrapolated FA synthesis *de novo* of 40 kg adipose tissue during the optimal conditions in the present study. Considering this, and also the dilution of *de novo* synthesized FA with FA from the intracellular lipolysis, the correlation between adipose tissue metabolism and serum TG cannot possibly be explained by a direct relationship. It is more likely that the correlations found in the present investigation might be explained by coincident increases in liver synthesis of FA (71, 72), TG (31, 71, 77) and lipoprotein-protein (30), and in adipose tissue synthesis of FA, all changes being induced by the high-carbohydrate feeding. The changes in serum lipids will be further discussed in a separate paper reporting also the changes in separate lipoprotein fractions during high-carbohydrate feeding (42).

Basal plasma insulin as well as the sum of insulin values during oral glucose tolerance test were unchanged in the present study. Iso- or hypercaloric high-carbohydrate feedings are potent insulinogenic stimuli (33, 41, 66). However the present results agree with the study by Grey and Kipnis (41), who observed that in obese subjects given a hypocaloric high-carbohydrate diet (1 500

cal/day) for three weeks the basal insulin level did not differ significantly from control values.

Fatty acid synthesis

In the present study adipose tissue FA synthesis was measured in two different ways. First, glucose U- 14 C incorporation into FA by whole cell preparations was measured. This technique might give artificially low values of FA synthesis because of dilution of radioactive precursor but has the advantage that at least some of the regulatory mechanisms for FA synthesis *in vivo* are preserved also *in vitro* (33).

Labelled acetate and citrate incorporations into FA by an optimally fortified cytoplasmic assay system (79) were also measured. With this technique precursor dilution can be neglected. Presumably most factors inhibiting or promoting FA synthesis *in vivo* have, however, been abolished. The effect of substrate availability *in vivo* is also neutralized. Since the system is optimal, it may measure a precursor incorporation which approaches the overall capacity of the present enzymes in the sequence of FA synthesis.

If it is assumed that one glucose molecule results in two acetyl units, the molar ratio of cytoplasmic acetyl incorporation/whole cell glucose incorporation into FA should be >2 . The ratio was 1.7 ± 4.2 (mean \pm S.E.M., $n=36$) or 9.8 ± 2.6 ($n=20$) if incorporations smaller than 1 nmole/ μ g-aver were excluded from the calculations. This high mean ratio indicates that a considerable part of the enzyme capacity for FA synthesis was not "detected" with the whole cell technique because of precursor dilution, inhibiting factors or substrate deficiency at the cytoplasmic acetyl-CoA level. Bray (21) demonstrated recently however that pyruvate is incorporated several times faster than glucose by human adipose tissue slices. Calculations from Bray's data show that, on a molar basis, pyruvate is incorporated into FA 0.7–17 times faster than glucose, indicating that some of the difference in FA synthesis between the intact cell system and the subcellular system might be due to precursor dilution or rate-limiting steps in glucose uptake or glycolysis. If 17 moles pyruvate are considered equivalent to 8.5 moles glucose, it seems probable that pyruvate incorporation into FA by the whole cell preparations in the present investigation would still have given a lower FA synthesis than

the cytoplasmic assay. A following paper (77) demonstrates that addition of palmityl(+)-carnitine to the cytoplasmic assay system does not increase the incorporation of acetyl units into FA. This fact and the calculations above indicate that the cytoplasmic system really approaches an optimal level.

FA synthesis measured with the whole cell technique increased 6–11 times and with the cytoplasmic assay 3–4 times during the high-carbohydrate feeding in spite of moderately negative energy balance. Glucose incorporations into CO_2 and glyceride-glycerol were not changed. Thus, when a hypercaloric effect is avoided, pathways involved in FA synthesis are more sensitive to a high-carbohydrate low-fat diet than those involved in CO_2 and glyceride-glycerol production. The results with whole cell technique agree with the concept that the FA synthesis in human adipose tissue is influenced by dietary changes, as proposed by Fessler et al. (33), Bray (20, 21), Kuo et al. (50), Melatti et al. (57), Goldrick and Hirsch (38) and Björntorp et al. (13).

On the other hand, the results with the subcellular assay system are not in agreement with the results of Shrago et al. (73, 74, 75, 76). According to these authors ATP citrate lyase [E.C.4.1.3.8] (citrate cleavage enzyme) is absent (73) or virtually absent (74, 75, 76) in human adipose tissue and this enzyme does not adapt to starving or refeeding (74, 75). Nevertheless Shrago et al. reported C^{14} -citrate incorporations into FA which were several times higher than the commensurate ATP citrate lyase activity (75, 76). There are two possible explanations of this discrepancy. 1) In human adipose tissue carbon from citrate is not incorporated into FA via the ATP citrate lyase step. In rat mammary gland (83), pigeon liver (8) and chicken liver (84) only C1 and C2 of the citrate molecule are incorporated into FA via acetyl-CoA, while the remaining oxaloacetate is not converted into FA in significant amounts by cytoplasmic systems (8, 83). Quantitatively important incorporation of cytoplasmic citrate into FA via other routes than ATP citrate lyase does not seem to have been reported for any tissue. 2) The assay conditions for ATP citrate lyase are not appropriate for human adipose tissue. Explanation no. 2 seems to be more plausible since the present investigation, as well as works by Shrago et al. (75, 76),

demonstrated that labelled citrate is readily incorporated into FA even when intramitochondrial transformation of citrate is excluded. Furthermore, attempts in this laboratory have failed to obtain linear conditions and sensitivity sufficient for determination of ATP citrate lyase and acetyl-CoA synthetase in human adipose tissue by the hydroxamate method (56) (unpublished observations). Contrary to the conclusions of Shrago et al., the present investigation has indicated the presence of ATP citrate lyase [E.C.4.1.3.8] in human adipose tissue, and also demonstrated that, *in vitro*, the enzyme capacity for citrate incorporation is at least as large as the capacity for acetate incorporation into FA if optimal conditions are used for both precursors (Citr 110 and Ac 30, Table V).

In earlier investigations several authors have taken adaptive changes of enzymes in the sequence of FA synthesis as circumstantial evidence that these enzymes have a regulatory function in the synthesis. As discussed in a following hypercaloric study (77) the supply of acetyl units is most probably rate-limiting for FA synthesis in rat as well as human adipose tissue. Nevertheless an almost maximal enzymatic capacity measured with the cytoplasmic assay system was reached already on the 4th day while a significant increase of glucose incorporation into FA was not obtained until the 23rd day. This pattern, with elevation of the enzymatic capacity preceding increases in FA synthesis, seems to be unusual since in rat adipose tissue (68) the increasing activities of acetyl-CoA carboxylase and of the FA synthetase complex are parallel with the increasing FA synthesis, and in rat liver (35) and pig adipose tissue (60) the increasing activity of ATP citrate lyase [E.C.4.1.3.8] follows on the increased FA synthesis during refeeding.

The present investigation demonstrates that the *de novo* FA synthesis probably is of little quantitative importance for glucose uptake and FA assimilation during basal conditions and after 22 days of moderately hypocaloric high-carbohydrate feeding, even if *in vivo* synthesis might reach the upper enzymatic limitation of FA synthesis as indicated by the cytoplasmic assay system. The maximal mean synthesis of 40 nmoles of acetyl unit incorporated into FA/g TG and hour (Table V) corresponds to about 1.2 g palmitic acid synthesized by 40 g adipose tissue during 24 hours.

Assuming that 1 mole glucose results in 2 moles acetyl unit this corresponds to 3.5 g glucose. The obvious hazards of such extrapolations are diminished since the cytoplasmic assay system gives values of the same magnitude when adipose tissue biopsies from the epigastric, hypogastric, femoral or gluteal regions or from the omentum are used (78).

All types of subcellular incorporation were greater in large than in normal-sized fat cells, as calculated by analysis of variance (Table VI). Ctr 30 and FA-max demonstrated an increasing correlation with fat cell size during the course of high-carbohydrate feeding (Table VII). The lack of correlation between FA synthesis and fat cell size before high-carbohydrate feeding might be due to disturbing host factors. In experiments with fat cells of different sizes isolated from the same adipose tissue Björntorp and Sjöström (15) demonstrated positive correlations between fat cell size and several metabolic activities including FA synthesis. Subjects on high-carbohydrate diets had positive correlations between fat cell size and metabolic activities. Host factors, diet and, as also shown in the present work, age (Table VII) thus influence the activity of fat cells in relation to their size.

Melatti et al. (57) demonstrated positive intercorrelations between glucose incorporation into CO_2 , glyceride-glycerol and FA in omental adipose tissue taken during abdominal surgery. Before high-carbohydrate feeding most of these intercorrelations were not significant in the present study using subcutaneous adipose tissue of overnight fasting women, but during the course of high-carbohydrate feeding all these intercorrelations became positive and significant. The significantly increasing sensitivity of FA synthesis to insulin during the course of high-carbohydrate feeding (Fig. 2) further stresses the importance of strictly defining the feeding state of donors of adipose tissue in all metabolic experiments.

ACKNOWLEDGEMENTS

This investigation was supported by Bofors AB, Mölndal, and by grants from the Swedish Nutrition Foundation, Nordisk Insulinfond, Torbjörn Nilsson Fund for Medical Research, and the Swedish Medical Research Council (grants B71-61P 1231-01 and B70-19X 231-07B).

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ARRHYTHMIA PROPHYLAXIS WITH PROCAINE AMIDE. PLASMA CONCENTRATIONS IN RELATION TO DOSE

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Abstract. Forty patients with suspected or confirmed acute myocardial infarction have been treated with procaine amide orally because of ventricular arrhythmia. The patients are divided into three groups with respect to doses and dosage intervals. Frequent determinations of the concentration of procaine amide in plasma were performed by fluorometric method. Plasma concentrations between 4 and 6 $\mu\text{g/ml}$, which have been reported to be the therapeutic range, were achieved only with one of the dose schedules employed, i.e. total daily dose of 50 mg/kg b.wt., administered in divided doses every 3 hours. However even 4-hour administration interval may give rather acceptable plasma concentrations. 1 patient with cardiac and renal insufficiency the dose must be individually reduced.

The drug of choice nowadays in the treatment of active ventricular arrhythmia (e.g. premature ventricular extrasystoles, ventricular tachycardia) during the initial phase of acute myocardial infarction is *lidocaine* which, however has to be administered parenterally. *Procaine amide* is therefore generally used in Sweden for oral arrhythmia prophylaxis in the postinfarction period.

Pharmacokinetic and pharmacodynamic studies of procaine amide have been performed both in healthy subjects and in cardiac patients (1, 2, 8, 9, 10) with fairly similar results in both groups. The absorption of procaine amide is mostly complete. A maximum plasma level is reached 60-90 min after oral administration, whereupon the concentration declines with a half-life of about 3.5 hours. Approximately 15% is bound to plasma

proteins at therapeutic plasma levels. The drug reaches higher concentrations in parenchymatous organs than in plasma. Approximately half of the administered dose is excreted unchanged in the urine and 2-10% is excreted as para-amino-benzoic acid or its conjugates. Dreyfuss (5) has shown that the major metabolite is N-acetylprocaine amide, which also agrees with our own observations (Colliste et al., unpublished data). Mark et al. (10) studied the plasma concentrations in ten healthy subjects who took procaine amide for 10 days in a dose of 0.75 g 4 times daily. Steady state was attained on the third day with a plasma concentration which varied from 3 to 9 $\mu\text{g/ml}$. Kayden et al. (6) and Koch-Weser and Klein (8) achieved steady state after only 12-24 hours using somewhat larger doses and more frequent administrations, and after only 6-9 hours if a loading dose was given (8).

Both Kayden et al. (6) and Bellet (1) recommended 3-6 g/day in 3 divided doses as an appropriate dosage for oral procaine amide in the treatment of ventricular arrhythmias. According to Bellet (1), however 1-2 g/24 hours with a 6-hour administration interval should be sufficient to prevent the recurrence of ventricular extrasystoles.

Kayden et al. (7) maintained that an adequate plasma concentration for procaine amide is 10-20 $\mu\text{g/ml}$. However Bellet (1) reported that he had obtained a satisfactory therapeutic effect in most patients with plasma concentrations around 5 $\mu\text{g/ml}$. Bigger and Heissenbuttel (2) reported a therapeutic range of 5-10 $\mu\text{g/ml}$. A comprehensive and thoroughly executed double-blind study of the relationship between dosage, plasma con-

A preliminary account of this work has been presented previously (4).

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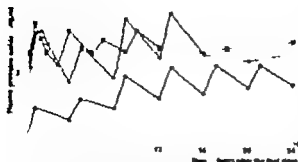


Fig. 1 Mean plasma concentration before and 1 hour after each dose in groups A (●—●), B (○—○) and C (■—■). — open plasma concentration before each dose only. The therapeutic range of 4–6 µg/ml suggested by Koch-Weser et al. (9) is given.

centration and therapeutic effect of oral procaine amide in the treatment of arrhythmias was published by Koch-Weser et al. (9). Seventy patients with acute myocardial infarction (AMI) but without complicating congestive heart failure or atrio-ventricular block were treated either with a placebo ($n=33$) or procaine amide ($n=37$) in 2–4 g doses/24 hours using a 3-hour administration interval after an initial loading dose of 1 g. ECG recordings and determinations of plasma levels were made at frequent intervals.

It was found that procaine amide significantly reduced the incidence of active ventricular arrhythmias. The therapeutic effect was related to the concentration of procaine amide in plasma. Partial protection against ectopic impulse formation was noted at a plasma concentration of 2–µg/ml and effective protection at 4–6 µg/ml. Side-effects were noted at plasma concentrations below 7 µg/ml. However three patients with plasma concentrations higher than 10 µg/ml had to be excluded from the study because of troublesome side-effects.

In a similar manner Koch-Weser and Klein (8) studied procaine amide treatment of different types of arrhythmia (136 cases of ventricular and 10 of supraventricular) in patients with a variety of basic disorders. Treatment was found to be ineffective in 50% of the cases when the plasma concentration was less than 4 µg/ml. An arrhythmia which endured at a plasma concentration of less than 8 µg/ml could be abolished at higher concentrations in only 10% of the cases. But there was also a concomitant increase in the frequency and severity of side-effects. At con-

centrations higher than 16 µg/ml haemodynamic disturbances and toxic effects on the electrical and mechanical performance of the heart were the rule and were commonly serious.

Wide interindividual plasma level variations were found after the same dose and amounted to as much as 350%. It was also found that the drug should be administered at more frequent intervals than had previously been recommended. During one administration interval a difference of 66% was found between the highest and the lowest plasma level at a dosage of 0.75 g 4 times daily. In order to avoid fluctuations in plasma concentration greater than 50% the drug had to be administered at intervals corresponding to its biological half-life. The half-life for procaine amide is approximately 3.5 hours. Therefore a maximum administration interval of 3 hours was recommended.

To summarize, Koch-Weser et al. (9) reported that the optimal plasma concentration was 4–6 µg/ml in the treatment of ventricular arrhythmias in conjunction with AMI. These authors also pointed out that the dose must be reduced in cases of renal insufficiency in which the renal excretion declines, and also in cases of congestive heart failure often resulting in a reduced distribution volume and possibly changes in drug metabolism.

Procaine amide 2.25 g daily divided into 6 doses has hitherto been the routine in our Coronary Care Unit (CCU). Inquiries made at several of Sweden's largest clinics disclosed that the most common daily procaine amide dosage is about 2 g, the individual doses being administered at intervals of 4–6 hours. Thus there is a certain amount of disagreement in the literature regarding the appropriate dosage. There is also a discrepancy between the normal dosage used by a number of Swedish hospitals and the dose advocated by Koch-Weser et al. (8, 9). That is why we studied the plasma concentration of the drug after various doses administered to patients with arrhythmias occurring in AMI. We also made certain observations on the relationship between the procaine amide concentration in plasma and its effects.

MATERIAL AND METHODS

A total material of 40 consecutive cases of suspected and proved AMI was planned for the present study. The pa-

Table 1 Patient material

OBS = observation cases without verified myocardial infarction

	Group A		Group B		Group C	
	AMI (n=10)	OBS (n=10)	AMI (n=7)	OBS (n=3)	AMI (n=6)	OBS (n=4)
Males	7	9	7	2	6	4
Females	3	1	0	1	0	0
Age (yr.)						
Mean	66	63	65	71	61	64
<45	0	1	0	0	0	0
>70	4	3	2	1	1	0
Previous myocardial infarction	4	3	2	1	0	1
Earlier history of arrhythmia	4	3	3	0	0	1
Onset of myocardial infarction						
During 48 h before study	8	8	5	3	3	2
Mean no. of h in these patients	37	32	36	33	35	30
Severity of myocardial infarction						
Mean highest ECGT	247	—	192	—	233	—
Mean highest CPK	385	—	499	—	815	—
Renal failure						
Creatinine clearance <80 ml/min	7	2	3	0	3	2
Creatinine clearance <30 ml/min	4	1	1	0	0	0
Hypotension during study ^a	2	0	2	0	1	1
Concurrent heart failure during study ^b	5	1	1	0	1	0
Mean dosage of procaine amide (mg/kg b wt/day) ^c	36	31	30	30	50	30

^aSystolic BP < 100 mmHg.^bSuspected cardiac insufficiency symptoms leading to treatment with digitalis and/or diuretics.^cGroups B and C also received an initial loading 1.0 g oral dose.

Urota were collected during six months in 1971-72. In the course of these six months 248 patients with suspected AMI are admitted to the CCU of the Department of Internal Medicine at the University Hospital of Linköping. Seventy-six patients received lidocaine infusion for ventricular arrhythmias appearing within 48 hours after the acute infarction. The latter patients were assessed for inclusion in the study. The following patients were excluded: patients with pulmonary oedema or cardiogenic shock (7 cases), AV block II to III or bradyarrhythmia without pacemaker management (7 cases), known and pronounced renal insufficiency (1 case), history of asthma or procaine amide hypersensitivity (2 cases). Nor did patient take part in the examination more than once even if suspected reinfarction occurred (2 cases). Ten patients were already on procaine amide therapy on admission. One patient was excluded because another treatment had to be initiated due to therapeutic failure. Two patients had total of only 2-3 extracardiac extrasystoles on admission, which led to lidocaine therapy. Lidocaine was subsequently withdrawn as continued recurrence prophylaxis as not felt to be indicated. Also these patients are excluded from the study. Six patients are not included because of technical errors. One patient is excluded because of side-effects and five because of pronounced absorption delay (see below).

Thus 40 (53%) of the originally 76 patients with ventricular arrhythmias calling for treatment according to accepted criteria (3) were ultimately included in the study. They received conventional, i.e. lidocaine infusion

until lapse of 24 hours without arrhythmic complications. All patients are continuously monitored by oscilloscope. An ECG recording was obtained from tape loop recorder with playback facility whenever arrhythmia is noted. A 12-lead ECG was routinely recorded at least once a day for analysis of the PQ, QRS and QT intervals. BP was checked at least every three hours. Electrolytes, acid-base balance, Hb, haematocrit and liver function were analyzed for each patient. Renal function was evaluated by creatinine clearance (except for one patient) and serum creatinine (all patients).

The material was divided into three groups according to the procaine amide dose and the administration interval. The patients were also divided into the categories proved acute myocardial infarction and observation cases. The patients admitted on suspicion of myocardial infarction which could not be verified.

Group A comprising 20 patients, 10 of whom had AMI, received oral procaine amide in 375 mg dose 1-4-hour intervals (2.25 g/day) after 4 hours of lidocaine infusion without development of ventricular arrhythmia. No loading dose was given. The infusion rate for lidocaine was successively reduced and lidocaine was withdrawn 4 hours after the start of procaine amide treatment. Blood samples for determination of procaine amide concentration are taken immediately before and 1 hour after each procaine amide dose for the first 24 hours (Fig. 1). Blood samples were also drawn from 3 patients every 30 min for 8 hours (Fig. 4).

Group B comprised 10 patients, 7 of whom had AMI,

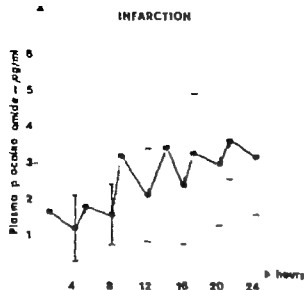


Fig. 2. Group A. Plasma concentration in 10 patients with AMI and 10 observation cases (mean \pm S.D.)

3 were observation cases. These patients received an oral loading dose of 1 g procaine amide and thereafter 50 mg/kg/24 hours with a dose interval of 4 hours. Lidocaine therapy was terminated without gradual reduction one hour after the loading procaine amide dose. Blood samples for determination of plasma concentration were taken every 30 min during the first 16 hours and then immediately before and one hour after each new dose for the next 11 hours. Blood samples were later taken only immediately before each dose for the remaining 7 hours in order to verify the attainment of steady state levels.

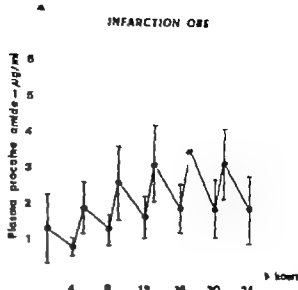
Group C comprised 10 patients, 6 of whom had AMI, 4 were observation cases. The patients in this group all received the same procaine amide regimen as group B but the administration interval was diminished to 3

Measurement of plasma concentration

5 ml blood was taken in heparinized tubes and centrifuged within one hour. The plasma was withdrawn and kept frozen at -20°C until analysis, which was done within 12 weeks. Procaine amide is not readily decomposed by plasma enzymes (10).

The plasma concentration of procaine amide was determined either by spectrophotometry (10) or spectrofluorometry (8). Because of the toxic properties of benzene, we used toluene as the extractant, obtaining the same results.

After double extraction in alkaline and acid environments, respectively the substance was dissolved with Britton-Marshall reagent. The spectrophotometer was read at 540 m μ . With the fluorometric method readings were made at the activating wavelength of 295 m μ and the fluorescence wavelength of 360 m μ . The latter method is faster than the former and was mostly used. Close agreement between the two methods was noted.



The reliability of the methods was tested through the analysis of 15 samples taken from a plasma pool with known procaine amide content (S.D. \pm 3%). Concentrations down to 0.5 µg/ml can be measured with these methods. In agreement with other authors, we found that 98 \pm 2% of the procaine amide is recovered from plasma (8, 10).

In order to test the specificity several drugs which theoretically might interfere with the staining procedure were tested, e.g. sulfanilamide, mephentermine, carbamazepine, quinidine and lidocaine. Sulfanilamide produced staining but was not extracted from plasma in the extraction procedure. The remaining compounds showed no staining properties.

RESULTS

Table I presents the characteristics of the 40 patients included in the study. The patients with myocardial infarction in group A appeared to display more frequent signs of deranged renal function and cardiac insufficiency than the patients in the other groups, but otherwise the groups seem to be comparable.

The plasma concentration of procaine amide was considerably lower in group A patients than in the other two groups. No patient with normal renal function (creatinine clearance exceeding 80 ml/min) had a plasma concentration within the therapeutic range defined by Koch-Weser et al. (9). As a rule the concentration one hour after administration did not even amount to 4 µg/ml (Fig. 1). There was no significant dif-

ference between patients with and without myocardial infarction as regards the mean and the S.D (Student's *t*-test) (Fig. 2). All patients with normal renal functions had achieved a steady state plasma level within 12 hours, which was not the case, however for patients with renal insufficiency. In cases of severe kidney damage, steady state was not achieved even after 24 hours. Fig. 3 shows examples of plasma concentration curves for one patient with normal and two with deranged renal function.

Blood samples taken every 30 min for 8 hours in two patients disclosed that maximum plasma concentration was reached 30–60 min after administration. Both patients appeared to have a typical absorption rate, with a fairly constant interval between the time of administration and maximum plasma concentration (Fig. 4). The patients in group B all had higher plasma concentrations, as shown in Fig. 1. The wide fluctuations in plasma concentration led to values less than 4 µg/ml during about 1 hour around each administration. The fluctuations were considerably less pronounced in group C with 3-hour administration intervals and generally ranged from 4 to 6 µg/ml (Fig. 1).

Failure of therapeutic effect

Group A In 6 cases out of 20 4 being patients with myocardial infarction and 2 observation cases, there was a therapeutic failure, i.e. ventricular arrhythmia developed which called for treat-

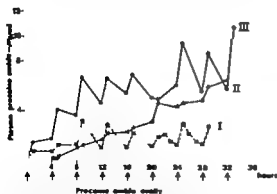


Fig. 3 Plasma concentration curves for 3 patients with and without renal insufficiency. Pat. I, II and III: dose 33, 38 and 32 mg/kg/day creatinine clearance 142, 45 and 29 ml/min, respectively.

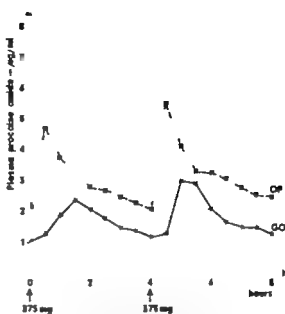


Fig. 4 Plasma concentration curves for 2 patients in group A (4-hour administration interval) after 30 hours of procaine amide treatment. GO: dose 33 OP: 32 mg/kg/day.

ment. Three of these patients displayed therapeutic failure despite plasma concentrations from 4.0 to 7.5 µg/ml. In two patients the plasma concentrations were only 0.4 and 1.4 µg/ml, respectively. Unfortunately in one case no plasma sample was taken during the arrhythmic episode.

Group B One patient with myocardial infarction showed therapeutic failure despite a plasma concentration of 6.2 µg/ml.

Group C Two patients with myocardial infarction in this group also presented therapeutic failure. The plasma concentrations during the arrhythmia were in these cases 4 and 7.5 µg/ml, respectively.

ECG changes

The 12-lead ECG recorded routinely before, during and after the study was analyzed. ECG changes which might possibly be due to procaine amide therapy (prolonged PQ, QRS or QT interval) were noted in only 6 patients. Most likely however procaine amide was not the cause as none of these patients had a very high plasma concentration. All of them had also extensive infarction, which may explain the ECG changes.

INFARCTION

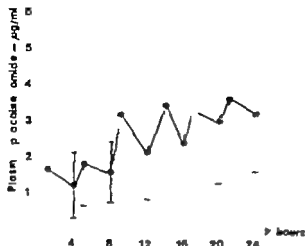


Fig. 2 Group A. Plasma concentration in 10 patients with AMI and 10 observation cases (mean \pm S.D.).

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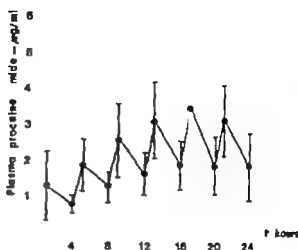
Termination of plasma concentration

5 ml venous blood were taken in heparinized tubes and centrifuged within one hour. The plasma was withdrawn and kept frozen at -20°C until analysis, which was done within 12 weeks. Procaine amide is not readily decomposed by plasma enzymes (10).

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INFARCTION OBS



The reliability of the methods was tested through the analysis of 15 samples taken from plasma pool in a known procaine amide content (S.D. $\pm 3\%$). Concentrations down to 0.3 $\mu\text{g/ml}$ can be measured with these methods. In agreement with other authors, we found that $96 \pm 2\%$ of the procaine amide is recovered from plasma (8, 10).

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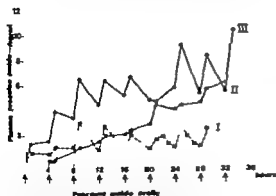


Fig. 3. Plasma concentration curves for 3 patients with and without renal insufficiency. Pats. I, II and III dose 33, 33 and 32 mg/kg/day creatinine clearance 142, 45 and 29 ml/min, respectively.

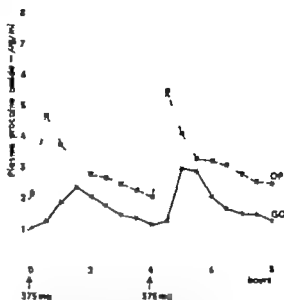


Fig. 4. Plasma concentration curves for 2 patients in group A (4-hour administration interval) after 20 hours of procaine amide treatment. GO: dose 33 OP: 32 mg/kg/day.

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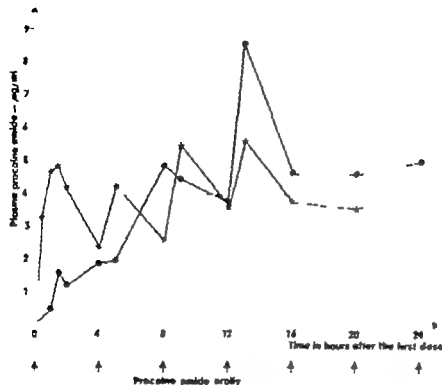


Fig. 5 Plasma concentration for one patient with asterisk (*) and mean plasma concentration in group B (—○—) after 1.0 g initial oral loading dose followed by administration of 30 mg/kg/day at 4-hour intervals. — plasma concentration before each dose only

Effect on blood pressure

One patient with a large myocardial infarct in group C had to be excluded from the study because of a drop in BP from 140/90 to 95/80 and at the same time impairment of her general condition. The plasma concentration of procaine amide, however did not exceed 6.0 µg/ml. In two other patients in group B there was a transient BP drop to 60 and 90 mmHg after the loading dose was given. The maximal plasma concentrations during these episodes were 9.1 and 9 µg/ml, respectively. A moderate 10–20 mmHg drop in systolic BP was also observed in our other cases, two in group A and two in group C. There was no definite relationship between the fall in BP and concentration of procaine amide in plasma in these cases. Nor were there any other signs of left ventricular failure which could definitely be ascribed to the procaine amide therapy.

Other side-effects

Mild gastrointestinal distress occurred in a few cases, mainly consisting of nausea in conjunction with the tablet administration. In no case had the therapy to be discontinued and no further side-effects were noted.

Mortality

Four of the patients in the study succumbed, three in group A and one in group C. Two patients died of ventricular fibrillation one of progressive left ventricular failure probably due to papillary muscle rupture and attendant mitral insufficiency (unfortunately no autopsy was performed). These three patients had plasma concentrations ranging from 4 to 8 µg/ml. Another patient died of rupture of the heart. This patient had a high plasma concentration with a maximum value of 10.5 µg/ml, but neither haemodynamic disturbances nor any arrhythmia were noted prior to the rupture. Autopsy was performed in three of the four cases and disclosed extensive myocardial infarction.

DISCUSSION

This study shows that the procaine amide dosage regimen generally practised in Sweden, approximately 2 g/day divided into 4–6 doses, results in very low steady state plasma concentrations which never rise to the optimum level advocated by Koch-Weser et al. (9) i.e. 4–6 µg/ml. This plasma level is only achieved after a dose of approximately 50 mg/kg/day with a maximum administration interval of 3 hours. Even a 4-hour

interval seems, however, to give acceptable plasma concentrations with the exception of 30–60 min just around each administration.

Even though a 2.25 g/day dose of procaine amide is far too small for the ordinary patient, it proved to be too large in certain cases with renal insufficiency resulting in successively rising plasma concentrations up to toxic levels. A drastic reduction in dose is required in these cases. Dosage is further complicated by the fact that renal dysfunction also may vary during the course of an infarction with changes in the patient's general circulatory status. Koch-Weser and Klein (8) reported that the distribution volume of procaine amide varies with these conditions. Probably interindividual differences in drug metabolism are also important. The gastrointestinal derangement which often occurs in cases of AMI presents another problem. In five of our patients in groups B and C the anticipated rise in plasma concentration after the first doses of procaine amide failed to appear. All of these patients had previously suffered from vomiting and some of them still complained of nausea at the time when the procaine amide was given. Their initial drug absorption was probably severely impaired. But their plasma concentrations rose quickly to high levels (Fig. 5) after about 10 hours in parallel with an improvement in general condition. Probably the absorption of the tablets, accumulated in the gastrointestinal tract, had begun at this time. Thus high doses present a risk for the infirm and nauseated patient, initially giving poor arrhythmia protection, and ultimately leading to toxic plasma concentrations of the drug. Parenteral administration would appear to be preferable in such cases.

Only routine oscilloscope monitoring was used without an arrhythmia detector. This means that a number of arrhythmias may possibly have been missed. However an established therapeutic failure was noted in 9 out of 40 cases, despite verified plasma concentrations of 4–7 µg/ml in six of these cases. All of these patients had extensive infarction, which was also complicated in one case by left ventricular insufficiency.

In conclusion, the prophylactic treatment of arrhythmias with procaine amide appears to be effective in most cases, but may be difficult to accomplish in the individual case. The dosage regimen generally adopted in Sweden, with the

administration of approximately 2 g/day divided into 4–6 doses, is insufficient. Patients with normal renal function and without circulatory disturbances require approximately 50 mg/kg/day with a maximum administration interval of 3 or possibly 4 hours. Large interindividual differences in the plasma concentration are found. An individually tailored dose reduction is called for in cases of cardiac or renal insufficiency. Frequent determinations of the plasma concentration are especially desirable in these cases. Infirm patients with suspected absorption derangement should receive parenteral administration.

The use of a drug such as procaine amide, adequately administered every 3 hours for 24 hours a day is difficult to manage. There is a considerable risk of over-dosage in cases of renal insufficiency and as arrhythmia is often its first manifestation its detection is often difficult and lethal complications may develop. Therefore, a comparative analysis of the therapeutic effect of procaine amide and other antiarrhythmic drugs with a longer half-life is obviously needed.

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PERICARDIAL EFFUSION IN MAN

Haemodynamic Adaptation after Gradual Pericardiocentesis in Patients with Varying Circulatory Impairment

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Abstract. The study describes the haemodynamic events in five patients with moderate pericardial effusion before, during and after gradual withdrawal of the pericardial fluid. The most marked changes occurred in all cases after the first portion had been aspirated. In general this resulted in an increase in stroke volume and arterial blood pressure although with great individual variations. The intrapericardial, right atrial and left ventricular end-diastolic pressures decreased in all patients. In two cases with constrictive components these latter two pressures, however, remained at pathologically elevated level at the end of the investigation. In the other cases there was an almost parallel fall in pericardial pressure and in diastolic intracardial pressure in the right as well as the left heart. The described method with simultaneous recording of intrapericardial and intracardial pressure can be used to distinguish between two types of pericardial disease at an early stage of the investigation. One type is characterized by fluid effusion only while the other has the effusion superimposed upon more pronounced anatomical changes of the visceral layer of the pericardium and possibly also of the myocardium.

Virtually any process causing pericardial damage may lead to accumulation of fluid in the pericardial space and give rise to the clinical picture of heart tamponade (1-10, 19).

The haemodynamic events in connection with artificially produced heart tamponade have been investigated repeatedly in animals (3-6, 8, 12-15, 17-18, 23-25). These studies show that increasing intrapericardial pressure causes a successively diminishing venous return and cardiac output and increasing diastolic intraventricular pressures. Related to the respiratory cycle a paradoxical pulse may appear (2, 7, 9, 11).

In few patients have haemodynamic studies

been performed during pericardiocentesis and removal of pericardial effusion (19, 21, 22).

The present study comprises a detailed haemodynamic study in five patients with pericardial effusion with varying degree of circulatory impairment. Observations were made before, during and after gradual withdrawal of pericardial fluid. Cardiac output and pressures in the pericardial sac, right atrium, pulmonary artery, left ventricle and in a peripheral artery were recorded according to a fixed protocol.

PATIENTS

Four patients with acute non-traumatic pericardial effusion were examined as soon as possible after the diagnosis had been established. The fifth patient, who was investigated had chronic pericardial effusion. The diagnostic procedures included physical examination, ECG, roentgenological examination, echocardiogram and phonocardiogram. Clinical data appear in the case reports.

METHODS

All patients were examined without premedication. A polyethylene catheter (PE 205) was placed percutaneously in the right atrium via femoral vein. A smaller catheter was introduced into the pericardial sac via a percutaneous puncture between the xiphoid process and the left costal margin (16, 20). One catheter was introduced in the pulmonary artery via cubital vein. A polyethylene catheter was placed in the brachial or femoral artery using the Seldinger technique. Another polyethylene catheter was placed in retrograde direction in the left ventricle through percutaneous puncture of brachial or femoral artery.

The respiratory phases are recorded using thermoclement registering the temperature of the expired air below the nostrils. A precordial differential ECG was also recorded. The pressures are measured by Statham

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Table I Individual haemodynamic data

PP—pericardial fluid, HR—heart rate, CO—cardiac output (l/min), SV—stroke volume (ml), P—pressure, Peric.—pericardium, RA—right atrium, LVEDP—left ventricular end-diastolic pressure, PA—pulmonary artery, BA—brachial artery, FA—femoral artery; all pressures given in mmHg

Case no.	Aspirated PP (ml)	HR	CO	SV	Peric. (mean)	P _{RA} (mean)	LVEDP	P _{PA} (syst./diast./mean) ^a	P _{RA} or P _{BA} (syst./diast./mean) ^a
1	II	68	6.0	88	5	7	11	38/14 24	149/64 89
	250	72	6.5	90	1	7	9	40/12 24	164/66 93
	370	72	6.5	90	2	6	10	43/13 25	164/66 102
2	0	94	4.9	52	6	8	9	26/17 23	99/53 73
	250	101	7.0	69	0	4	5	35/14 23	112/60 77
	455	100	5.8	58	0	2	4	31/14 21	104/56 74
3	0	76	6.9	90	4	5	9	19/8 13	91/53 77
	250	76	8.6	114	1	4	12	21/6 13	98/57 76
	500	74	8.4	114	—1	2	9	19/4 12	98/58 75
	750	70	7.8	111	—1	1	8	19/7 12	98/58 79
	850	72	7.6	106	—2	1	6	15/6 10	100/59 78
4	0	110	3.7	33	13	14	20	43/23 30	136/74 89
	250	108	6.4	59	3	8	12	50/21 29	138/77 86
	500	108	5.7	53	0	8	13	46/18 28	125/62 90
	735	109	4.7	43	0	9	12	40/17 25	125/66 86
5	0	78	9.7	125	2	2	7	19/7 12	142/75 100
	250	84	10.2	123	—2	0	6	18/5 11	146/78 100
	500	82	10.0	123	—3	—2	3	17/4 8	149/83 104
	750	89	8.9	100	—4	—1	3	16/3 8	149/83 96
	1 000	96	9.8	102	—8	—3	1	16/4 8	143/78 96

Mean values given in *italics*.

Pb 23 db transducers, amplified by Beckman dynograph R and recorded with an Ultravette writer on a photo paper.

The pressures in the pericardial sac, right atrium, pulmonary artery and left ventricle were recorded simultaneously. The same amplifications and the same zero lines were used for all the recordings. The pressure in the left ventricle was also recorded, using high pressure standard simultaneously with that of the peripheral artery and with the same amplification. All pressures were referred to level 5 cm below the angle of Louis.

All calculations of pressures are mean values from three respiratory cycles.

The cardiac output was determined by the dye dilution technique with dye injection in the pulmonary artery and blood sampling in peripheral artery using bromsulphalein as indicator. The pericardial fluid was examined with regard to packed cell volume, Hb and protein content. Bacteriological, virological and cytological examinations were also made on fresh specimens.

After all catheters had been placed in proper position the patient rested for 20 min. Recordings of the above mentioned parameters were then performed according to the following schedule: 1 Recording of pressures and estimation of cardiac output. 2 Withdrawal of 250 ml fluid from the pericardial sac lasting for about 5 min. 3 Recording of pressures. 4 Rest for 20 min. 5 Recording of pressures and estimation of cardiac output. 6 In the patients from whom more fluid could be withdrawn

from the pericardial sac the procedure was repeated from point 2 again. 7 After the last withdrawal all pressures were recorded four times at 10-min intervals during the last 40 min after all fluid had been removed. A final cardiac output estimation was then performed.

CASE REPORTS

Case 1

A 68-year-old man with mild rheumatic aortic valve incompetence. He had no symptoms from his heart disease. The patient was admitted because of low grade fever and cough that had lasted one week. During the last 12 hours he developed retrosternal pain not related to respiration. The patient was moderately dyspnoeic at rest and had some orthopnoea. On examination he had distended jugular veins and moderate hepatomegaly. The liver did not pulsate. Arterial blood pressure was 140/90 mmHg and no paradoxical pulse was observed. Heart auscultation revealed a moderate pericardial friction sound and a soft blowing systolic murmur grade III, best heard over the apex which was barely palpable. A chest X-ray revealed a diffuse heart enlargement (800 ml/m² BSA). Echocardiography showed an increased distance between the anterior chest wall and the heart.

Heart catheterization was performed in connection with pericardiocentesis. Totally 370 ml serous fluid

fluid was withdrawn. The examination of this fluid did not reveal the aetiological diagnosis. The haemodynamic data are shown in Fig. 1 and Table I.

During the removal of the pericardial fluid the intrapericardial pressure first fell from 5 to 1 mmHg and then increased to 2 mmHg. The elevated right atrial pressure and the left ventricular end-diastolic pressure (LVEDP) decreased slightly. The systolic pressures in the brachial and pulmonary arteries rose somewhat, giving slight increases in pulse pressures. Stroke volume remained unchanged, while heart rate and cardiac output increased slightly. The arterial and left ventricular pressure curves showing an early diastolic dip and late diastolic plateau did not change during the procedure.

The patient recovered on prednisolone therapy and had no recurrence of pericardial exudation. The roentgenological heart size decreased from 800 to 670 ml/m² BSA.

Case

A 62-year-old woman with no heart symptoms previously in the last three weeks before admission she had attacks

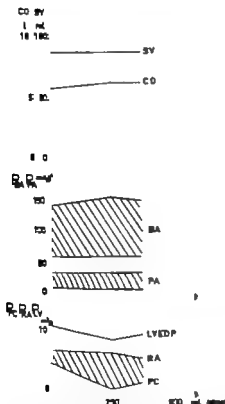


Fig. 1 Adaptation of haemodynamic parameters during gradual withdrawal of pericardial fluid in case 1. The areas between the systolic and diastolic pressure levels in peripheral artery as well as in pulmonary artery have been hatched, thus visualizing pulse pressures. Similarly the right heart filling pressure has been indicated by hatching the area between right atrial and pericardial pressure values. Symbols as in Table I.

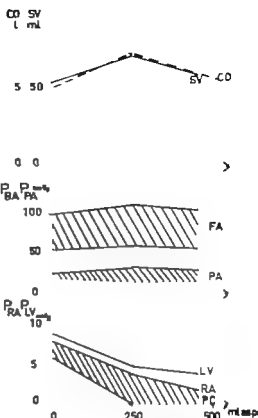


Fig. 2 Adaptation of haemodynamic parameters during gradual withdrawal of pericardial fluid in case 2. Symbols as in Table I.

of tachycardia presumed to be atrial fibrillation, although no ECG recording had been made during an attack. During the last week before admission she had had fever around 38–39°C and general muscle pains. She was in good general condition without chest pain or breathing difficulties. The arterial blood pressure was 120/90 mmHg and there was no paradoxical pulse. Heart auscultation revealed pericardial friction sound near the apex of the heart, but no other abnormalities. The ECG showed mild elevation of ST-T in precordial leads.

During the first two days in hospital retrosternal chest pain and dyspnoea appeared. A chest X-ray showed an increasing heart size and pulmonary venous congestion but no pleural fluid. The blood pressure remained unchanged, but the arterial pulse became paradoxical. Echocardiography showed an increased distance between the anterior chest wall and the heart. A pericardiocentesis as then performed in connection with heart catheterization. Altogether 455 ml seropalescent fluid was removed. Examination of the pericardial fluid gave no aetiological diagnosis. After about two weeks in hospital on anti-biotic therapy the patient was discharged with no remaining symptoms or signs.

The haemodynamic data in connection with the catheterization are shown in Fig. 2 and Table I.

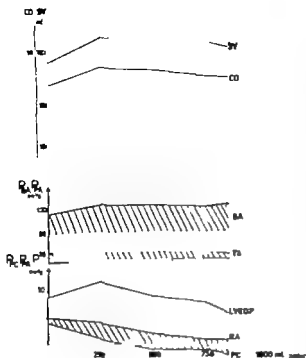


Fig. 3 Adaptation of haemodynamic parameters during gradual withdrawal of pericardial fluid in case 3. Symbols as in Table I.

During the removal of the pericardial fluid the intrapericardial pressure decreased from 6 to 0 mmHg. The right atrial pressure decreased successively from 8 to 2 mmHg and the LVEDP from 9 to 4 mmHg. The systolic pressures in the pulmonary and femoral arteries increased after withdrawal of the first 250 ml and then remained approximately unchanged. The pulse pressures both in the brachial artery and pulmonary artery increased moderately. Stroke volume and cardiac output increased concomitantly. The intraventricular pressure curves had neither early diastolic dip nor late diastolic plateau and remained unchanged during the procedure (except for the decrease in the level).

Case 3

A 27-year-old man who had been treated surgically because of mediastinal tumour that was shown to be due to fibrosarcoma. Postoperatively he was treated with radiation therapy. About four months after the operation he was back at work. One week later he experienced rather severe pain in the upper part of the abdomen and breathlessness. On examination he had facial oedema, distended jugular veins and hepatomegaly. The liver showed no pulsations. Heart auscultation revealed weak heart sounds and pericardial friction rub. The arterial blood pressure was 105/85 mmHg with paradoxical pulse. Chest X-ray revealed marked enlargement of the heart and bilateral pleural effusion. Echocardiography showed an increased distance between the anterior chest wall and the heart. Pericardiocentesis was performed and 1160 ml turbid fluid was withdrawn. Symptoms and signs of tamponade disappeared.

During the following two days there was an accumulation of pericardial fluid and pericardiocentesis was again performed. In connection with this the haemodynamic study was performed. The patient improved successively on antibiotic therapy and left the hospital. The definite cause of the pericardial effusion was never established. Haemodynamic data are shown in Fig. 3 and Table I.

During the withdrawal of fluid (totally 850 ml) there was gradual fall of the intrapericardial pressure from 4 to 2 mmHg and parallel decrease of the right atrial pressure and the LVEDP. The systolic arterial pressure increased. Stroke volume and cardiac output rose, but heart rate decreased somewhat. The configuration of the intracardiac pressure curves showed no diastolic dip or late diastolic plateau and remained unchanged.

Case 4

A 68-year-old man who during the last two years had had periodical left-sided pleural effusion of unknown aetiology. His heart was slightly enlarged and he was treated with digitalis. Suddenly he experienced dyspnoea at rest, orthopnoea and peripheral oedema, but no chest pain, and was then admitted as an emergency case. On examination he showed resting dyspnoea, the jugular veins were congested and he complained of pain in the left lower thoracic region. The arterial blood pressure was 120/80 mmHg, and there was paradoxical pulse. Auscultation of the heart was normal. The chest X-ray taken on admission showed a markedly increased heart size

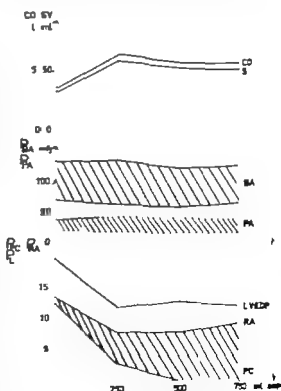


Fig. 4 Adaptation of haemodynamic parameters during gradual withdrawal of pericardial fluid in case 4. Symbols as in Table I.

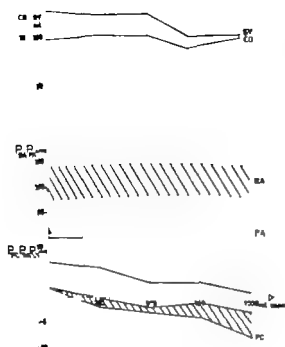


Fig. 5 Adaptation of haemodynamic parameters during gradual withdrawal of pericardial fluid in case 5. Symbols as in Table I.

and echocardiography revealed an increased distance between the anterior chest wall and the heart. Pericardiocentesis was performed in connection with heart catheterization. The haemodynamic data appear in Fig. 4 and Table I.

Totally 735 ml sanguinolent fluid was withdrawn. During the aspiration of fluid there was a successive drop in the intrapericardial pressure from 13 to 0 mmHg, but only slight decrease of the pressures in the right atrium and left ventricle. The intracardiac diastolic pressure drop is already maximal after the separation of the first 250 ml from the pericardial space. The systolic pressures in the pulmonary and brachial arteries increased somewhat with moderate increase in pulse pressure. The stroke volume and cardiac output, however rose markedly with unchanged heart rate. The intracardiac pressure curves showed no early diastolic dip or raised diastolic plateau and remained unaltered during the procedure. The patient successively improved, at first on prednisone therapy. Ten days after admission he developed sepsis. Antibiotic therapy did not influence his condition and he died ten days later.

Post-mortem investigation revealed fibrous constrictive pericarditis of the visceral pericardium with slight exudation. The pericardial sac contained 50 ml fluid. The left femoral vein as the site of septic thrombosis. The pulmonary arteries contained fresh and old pulmonary emboli. (The venous puncture had been made in the right femoral vein.) Investigation of the pericardial fluid revealed extracellular inclusion bodies. There was

no suspicion that the investigation had caused the septic thrombotic disease leading to his death.

Case 5

A 44-year-old mentally retarded woman without any history of cardiac decompensation. A routine chest X-ray showed pronounced heart enlargement (1110 ml/m² BSA). She had no symptoms. Physical examination revealed no jugular venous congestion and the heart sounds were weak and distant. A venous angiography showed marked distance between the heart chambers and the harts of the heart shadow. Pericardiocentesis was performed in connection with heart catheterization. The haemodynamic data appear in Fig. 5 and Table I.

A total amount of 1000 ml was withdrawn. The fluid was clear and straw-coloured. The pericardial pressure fell altogether 10 mmHg with concomitant decrease in right atrial pressure and LVEDP. The pulmonary and brachial arterial pressures changed only slightly.

Late during the gradual withdrawal there was fall in stroke volume and an increase in heart rate. The cardiac output remained unchanged.

The intracardiac pressure curves had no early diastolic dip or late diastolic plateau. After the pericardiocentesis there was refilling of the pericardial sac over few days. A pericardectomy was therefore performed. There were no signs of constriction at the operation. The aetiology of the pericardial effusion could not be determined. The roentgenological heart size decreased from 1100 to 530 ml/m² BSA after surgery.

DISCUSSION

Heart tamponade is a clinical diagnosis without any exactly defined limits of the circulatory impairment. Increasing amounts of fluid within the pericardial space will successively lead to a steep increase of the intrapericardial pressure, causing a pronounced impairment of circulation. The resistance to adequate filling of the right heart will lead to a clinical picture of heart tamponade of varying degree (10). This is characterized by a falling arterial blood pressure with decreasing pulse pressure, paradoxical pulse, a raised venous pressure and a falling cardiac output. Varying degrees of dyspnoea, orthopnoea, faintness and more or less diffuse chest or upper abdominal pain are symptoms that may develop rapidly in acute pericardial effusion.

In three of the four patients with acute pericardial effusion a rise in stroke volume occurred after the first 250 ml pericardial fluid had been removed. The following withdrawal of fluid caused a slight decrease of the stroke volume. The peripheral arterial pressure showed a similar pattern. The first aspirated portion is thus of greatest

haemodynamic Importance This has also been demonstrated earlier both in animals and man (19, 21, 22). In contrast the patient with chronic pericardial effusion (no. 5) at first reacted with largely unchanged arterial pressures and cardiac output. When more fluid was withdrawn (500–750 ml) a fall in stroke volume and arterial blood pressure occurred. This patient had low right atrial pressure to start with and this pressure decreased further to subatmospheric values, thus probably causing a diminished venous return. In spite of the diminishing stroke volume cardiac output was largely unchanged in this case due to increased heart rate. No major or systematic change in heart rate was observed in the other patients.

The pericardial pressures decreased in all patients by 3–13 mmHg. In three patients (nos. 2, 3 and 5) the decrease in the right atrial pressure and left ventricular diastolic pressure almost paralleled the fall in pericardial pressures. In one case (no. 4) there was a much more marked drop of the pericardial pressure than that of the right atrium and left ventricle during diastole though the pressure drops were almost equal after the first portion of fluid had been withdrawn. In this case the intracardial diastolic pressures thus remained at an abnormally elevated level (Fig. 4); the post-mortem investigation showed a fibrous constrictive pericarditis of the visceral layer of the pericardium. A similar pattern of the pressure levels was present in case 1.

Regarding the configuration of the intracardial pressure curves only one case (no. 1) showed an early diastolic dip and late elevated diastolic plateau. This is especially remarkable as this type of curve has been considered typical of pericarditis constricting the heart.

The difference in decrease of intracardiac—intracardial pressures indicates that two kinds of effusions might arise during pericardiocentesis in pericardial effusion. The pure tamponade—presumably characterized by effusion of fluid in the pericardium with little anatomical change of the visceral layer—will show a parallel reaction of the pericardial pressure, right atrial pressure and left ventricular diastolic pressure. The other kind of result occurs in patients who seem to have a symptom-producing pericardial effusion superimposed upon a pre-existing constriction due to perimyocardial changes. Thus pericardial fluid will cause impairment of the cardiac function irrespec-

tive of whether there is a concomitant visceral constriction or not.

Pulmonary arterial pressures did not change much in these investigations. This might be due to the fact that none of our patients had seriously impaired circulation. The initial pressures were also close to normal. Similar results have been obtained both in man (22) and animals (24).

"Paradoxical pulse" means an accentuation of the normal fall in arterial blood pressure during inspiration of more than 10 mmHg (2, 7, 9, 11). It has especially been described in cases of more pronounced heart tamponade. Only patients 2, 3 and 4 demonstrated this sign. Two of these patients (nos. 2 and 3) had only pericardial effusion, whereas the other (no. 4) had pericardial effusion superimposed upon a pericardial constriction. Paradoxical pulse has been said to be consistent with heart tamponade rather than with constriction (10). The reason why this was not the case in the present study might also be the rather slight impairment of cardiac function in our patients.

Analysis of haemoglobin or determination of packed cell volume in the fluid withdrawn from the pericardium were used to verify that the catheter was placed in the pericardial sac. Laboratory investigations of pericardial fluid using bacteriological, virological and cytological techniques have sometimes been of diagnostic value. In none of the present cases, however, did the examination reveal the aetiology of the pericardial effusion, nor did the post-mortem examination in one of the patients show more than non-specific inflammatory changes.

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ACID-BASE DISTURBANCES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract. In two years 417 cases of AMI without pulmonary disease were treated in CCU. During this period arterial blood gas samples were drawn by routine on the first day. The tape-stored data from the first day at the CCU were compared to pH, standard bicarbonate (SB) and base excess (BE) values. Low SB, BE and pH values were associated with high mortality and also with a high incidence of hypotension, shock and frank pulmonary oedema during the first day. Left heart failure without frank pulmonary oedema showed no such relation. As regards various arrhythmias, left bundle branch block was more common in patients with low pH, SB and BE values. Supraventricular tachycardia was more frequent finding in patients with low SB and BE values. High values of pH, SB and BE were not combined with an increased incidence of any arrhythmias.

It is well known that metabolic acidosis rapidly follows circulatory standstill. Some studies indicate that acidosis and also alkalosis may predispose to the development of ventricular fibrillation (VF) as well as interfere with its successful treatment (2, 4). Different opinions have been expressed, however concerning the prognostic value of measurements of acid-base balance in patients with acute myocardial infarction (AMI).

A significant association between low pH and mortality was found by Pücher and Nægle (14), and an increasing mortality rate with decreasing pH and standard bicarbonate (SB) has been described by Kirby (6). In other studies no significant relations were found between pH (3, 11) or SB (8) and the clinical severity of the infarction. An association has been described between acidosis and shock (8, 9, 12, 14) and between acidosis and frank pulmonary oedema (1).

Patients with AMI and arrhythmias are reported to have lower pH and SB than patients with AMI and sinus rhythm (8), but no such

relation has been found with respect to entricular arrhythmias in patients with uncomplicated infarctions (15).

The aim of the present investigation has been to study pH, SB and base excess (BE) in relation to mortality rate, incidence of hypotension, shock, left heart failure and various arrhythmias in 450 cases of AMI.

MATERIAL AND METHODS

During 1968-69 450 cases of AMI were treated at coronary care unit (CCU). There were 284 men (63%), mean age 63 years, and 166 women, mean age 71 years. The total hospital mortality was 21% and the CCU mortality 10.4%. During this period arterial blood gas analysis was performed by routine. A detailed description of the CCU and criteria for admission, discharge, therapy and diagnosis of AMI are given elsewhere (11, 15). On the first day pH was measured in 398 out of 450 episodes of AMI. SB was calculated in 349 and BE in 329 episodes. Thirty-three patients had coexisting pulmonary disease and are therefore excluded from the study and in 47 cases blood gas analysis had not been done during the first day.

The patients have been grouped according to pH, SB and BE as shown in Table I, and the different groups of patients have been compared with regard to age, sex, mortality rate and also to physical findings and the occurrence of various arrhythmias during the first day in the CCU. The age and sex distributions in the pH, SB and BE groups did not differ from those for the whole material.

Definitions

Left heart failure. Basal rales, third heart sound or chest X-ray findings of central aortic enlargement.

Frank pulmonary oedema. Patients with rales heard all over the chest in association with frothy sputum.

Hypotension. A systemic BP of 90 mmHg or below.

Shock. Hypotension in combination with clinical signs of shock such as cold skin, deterioration of sensation, oliguria.

Table I Grouping of patients according to pH, SB and BE

pH	No. of SB pts. (mEq/l)	No. of BE pts. (mEq/l)	No. of pts.
<7.35	22	<20 17	<(-) 5 39
7.35-7.40	101	20-21 34	(-) 3 - (+) 4 39
7.41-7.45	154	22-26 217	(-) 2 - (+) 0 86
7.46-7.50	67	>26 66	(+) 1 - (+) 2 70
>7.50	21		>(+) 3 62

Blood gas analysis

Arterial blood from the femoral artery was drawn in heparinized syringes. During the daytime samples were obtained within half an hour after admission. However in patients admitted after 4 p.m. the samples were not drawn until the next morning unless otherwise indicated. The pH was measured with pH-meter PHM 72 (Radiometer Copenhagen) with a glass electrode, capillary type. SB and BE were calculated according to the Siggaard-Andersen allometric nomogram. In the present study the normal values used were: pH 7.35-7.45 SB 22-26 mEq/l, BE ± 2 mEq/l.

Statistical method

All the statistical calculations have been made with χ^2 . The significances obtained have been expressed as follows: not significant $p > 0.05$ almost significant $0.01 < p < 0.05$ significant $0.001 < p < 0.01$ highly significant $p < 0.001$.

RESULTS

pH (Table I)

There were no significant differences in pH between different age or sex groups. Patients with pH < 7.35 had a higher hospital mortality rate than patients with pH ≥ 7.35 ($p < 0.05$). The difference in CCU mortality was however not significant.

Left heart failure without frank pulmonary oedema showed no relation to pH. However frank pulmonary oedema was more common in patients with pH < 7.35 than in those with pH ≥ 7.35 ($p < 0.01$). If patients with pH < 7.35 were compared to patients with pH ≥ 7.45 the difference is highly significant. Among patients with pH < 7.35 there were more with hypotension-shock as compared to patients with normal and/or high pH ($p < 0.05$).

Among the supraventricular arrhythmias, supraventricular bradycardia (SVB) and also supraventricular ectopic beats (SVEB) were more fre-

quent among patients with low pH as compared to those with high pH values ($p < 0.01$). Supraventricular tachycardia (SVT), atrial fibrillation and/or atrial flutter (AF) showed no significant relation to pH.

Among the ventricular arrhythmias the distributions of ventricular tachycardia (VT) and VF in relation to pH are similar with a tendency to higher incidence in acidotic patients. The difference is almost significant for VF. No significant differences were found in the incidence of VEB in the three pH groups. Nor were any such differences noted for the subgroups of VEB (monofocal multifocal, coupled and R on T). A higher incidence of asystole was noted in patients with pH < 7.35 than in those with pH ≥ 7.35 ($p < 0.001$).

No significant relationships were noted between pH and heart blocks of first (A V I) or second degree (A V II) or complete heart block (CHB) separately or together. Left bundle branch block (LBBB) was more common in patients with low pH values ($p < 0.05$) but also tended to be more common in patients with pH ≥ 7.45 as compared to those with normal pH values. This difference was, however not significant. Right bundle branch blocks (RBBB) though few in number showed the same pattern, but without any significant differences.

Table II Mortality and clinical data on day 1 in different pH groups (%) (N = 365)

	pH		
	<7.35 (n = 22)	7.35-7.45 (n = 235)	>7.45 (n = 108)
CCU mortality	18	9	6
Total hospital mortality	36	18	23
Hypotension-shock	36	18	15
Left heart failure without frank pulmonary oedema	30	60	63
Frank pulmonary oedema	27	6	10
SVEB	39	34	30
SVT	30	36	42
AF	27	16	26
SVB	32	22	8
VEB (during the first 12 h)	91	73	77
VT	43	34	32
VF	14	4	2
Asystole	27	5	3
1st degree heart block	13	18	11
2nd degree heart block	13	7	5
CHB	6	5	3
LBBB (including hemiblock)	32	13	18

Standard bicarbonate (Table III)

Low SB values were more common in patients >70 years than in those <70 years ($p < 0.05$). High SB values were more common in women than in men ($p < 0.05$).

There was a highly increased hospital mortality rate in patients with low SB values as compared to those with SB above 22 mEq/l ($p < 0.001$), and a lower hospital mortality in patients with SB above 26 mEq/l than in patients with normal SB values ($p < 0.05$). The hospital mortality rate in patients with SB <22 mEq/l was 34% in those with normal SB 17% and in those with SB above 26 mEq/l 8%. The CCU mortality rate was not as clearly related to SB as the hospital mortality rate. A significantly increased CCU mortality was only noted for patients with SB values less than 20 mEq/l when compared to patients with normal values.

SB like pH, showed no significant relation to left heart failure without frank pulmonary oedema, while frank pulmonary oedema was more common in patients with low SB than in patients with SB >22 mEq/l ($p < 0.001$). Hypotension-shock during the first day was more common in patients with low SB than in patients with normal and high values ($p < 0.001$).

SVT was seen more often in patients with low SB values than in those with normal or normal

Table III. Mortality and clinical data on day 1 in different SB groups (%) (N=336)

	SB (mEq/l)		
	<22 (n=53)	22-26 (n=217)	>26 (n=66)
CCU mortality	17	6	6
Total hospital mortality	34	17	8
Hypotension-shock	40	15	9
Left heart failure without frank pulmonary oedema	49	62	55
Frank pulmonary oedema	28	4	12
SVEB	43	38	35
SVT	57	33	38
AF	23	18	20
SVB	15	22	14
VEB (during the first 12 h)	74	79	64
VT	40	35	29
VF	11	2	5
Asystole	8	5	5
1st degree heart block	11	10	14
2nd degree heart block	11	5	8
CHB	4	6	6
LBBB (including hemiblock)	30	12	14

Table IV. Mortality and clinical data on day 1 in different BE groups (%) (N=296)

	BE (mEq/l)		
	<(-)3 (n=78)	(-)2 (+)2 (n=156)	>(+)3 (n=62)
CCU mortality	17	6	6
Total hospital mortality	33	13	8
Hypotension-shock	33	13	8
Left heart failure without frank pulmonary oedema	55	61	55
Frank pulmonary oedema	21	6	11
SVEB	37	42	35
SVT	53	33	37
AF	24	18	21
SVB	21	22	15
VEB (during the first 12 h)	73	79	68
VT	40	36	31
VF	10	3	5
Asystole	5	6	6
1st degree heart block	9	10	13
2nd degree heart block	6	7	8
CHB	5	6	8
LBBB (including hemiblock)	24	12	16

and high values ($p < 0.01$), and AF was also seen more often in patients with low values than in those with normal values ($p < 0.05$). SVB and SVEB showed no relation to SB. As regards ventricular arrhythmias it was noted that the incidence of VEB and VT did not vary significantly in the different SB groups. VF but not asystole, was more common in the group with low SB values compared to those with normal and high values ($p < 0.05$).

With regard to disorders of A-V conduction SB like pH, showed no relation to A-V I, A-V II or CHB. LBBB was more common in patients with low SB values than in patients with normal values ($p < 0.001$).

Base excess (Table IV)

BE was related to age in the same manner as SB, i.e. low values were more common in patients >70 years old than in those <70 years. No relation was noted between BE and sex.

Hospital and CCU mortalities in relation to BE are presented in Fig. 1. There was a highly significantly increased hospital mortality rate and a significantly higher CCU mortality rate in patients with BE < -2 mEq/l as compared to those with BE > -2 mEq/l. The lower the BE value the higher the hospital mortality rate, 46% in



Fig. 1 CCU and hospital mortality rates in different groups according to BE levels.

patients with BE ≤ -5 mEq/l, 21% in patients with BE -4 to -3 mEq/l, 14% for BE -2 to ± 0 mEq/l, 11% for BE $+1$ to $+2$ mEq/l and 8% for BE > 2 mEq/l.

BE in relation to physical findings of circulatory disturbances during the first day is shown in Fig. 2. Left heart failure without frank pulmonary oedema was equally frequent in the different BE groups, while frank pulmonary oedema was more frequent in patients with BE ≤ -5 mEq/l than in the remainder of the patients ($p < 0.001$). Hypotension-shock was more common in patients with BE < -2 mEq/l than in those with BE ≥ -2 mEq/l ($p < 0.01$).

SVT was more common in patients with BE < -2 mEq/l as compared to those with BE ≥ -2 mEq/l ($p < 0.01$) while no relation between BE and SVEB or AP was noted. SVB was seen more often in patients with BE ≤ 0 mEq/l than in those with BE > 0 mEq/l ($p < 0.001$) while, as seen in Table IV there was no significant difference between those with low normal or high BE values.

As regards ventricular arrhythmias VEB were

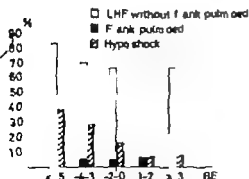


Fig. 2. Physical findings of circulatory disturbances during the first day in different groups according to BE levels.

equally distributed in the different BE groups. The incidence of VT was similar in the groups with negative BE values and higher in these groups than in patients with BE > 0 mEq/l ($p < 0.01$). There was an increased incidence of VF in patients with BE ≤ -3 mEq/l compared to those with BE > -3 mEq/l ($p < 0.05$). No significant differences were noted for ventricular asystole.

AV I, AV II and CHB were equally distributed among the groups with different BE values. LBBB was significantly more common in patients with BE ≤ -5 mEq/l than in those with BE > -5 mEq/l.

DISCUSSION

In the present study 6% of the patients had low and 24% high pH values. Corresponding figures for SB were 16 and 20% and for BE 26 and 21%. Some studies indicate that pH and SB values mostly are within the normal range in patients with AMI (1, 3, 8, 9, 11, 12, 13, 15). The results may be explained by the use of mean values in those studies. In fact some other studies of unselected patients with AMI show more patients with acidosis than ours (7, 12). The incidence of alkalosis was similar in the study of Kirby and McNicol (7) and in the present one.

Most authors agree that there is an association between acidosis and mortality in AMI (6, 7, 12). This is also true for the present study. There was an almost significantly higher hospital mortality rate in patients with low pH values and a highly significantly increased hospital mortality rate in patients with low BE and SB values as compared to patients with normal and high values.

Kirby and McNicol (7) found a hospital mortality rate of 75% in patients with SB < 15 mEq/l, which may be compared to a mortality rate of 53% in patients with SB < 19 mEq/l in the present study. In our study patients with pH < 7.35 had a hospital mortality rate of 36%. Kirby (6) found higher figures: 50% in patients with pH 7.30–7.35 and 89% in patients with pH < 7.30 . In the present investigation blood gas samples were not taken during the first day in 47 patients. The CCU mortality of those patients was 20% as compared to 10% in the whole group. This indicates that among the patients from whom no blood gas samples had been drawn there were

many who were seriously ill and who died before blood gas sampling.

Acid-base balance in relation to left heart failure has been studied several times (3, 6, 7, 8). Ljungström et al. (8) found that SB and pH were lower in patients with cardiac failure; Kirby and McNicol (7) found slightly reduced SB and normal pH in patients with left ventricular failure; Kirby (6) found normal SB and pH mean values, and Fillmore et al. (3) found normal pH in patients with left heart failure. In the present study left heart failure without frank pulmonary oedema showed no relationship to pH, SB or BE. Frank pulmonary oedema, on the other hand, was related to low pH and SB values and also to BE values < -5 mEq/L. However most patients with frank pulmonary oedema had developed this complication before admission and in most of these cases arterial blood gas samples were taken in connection with the initial treatment of the patients. In the present study it is also evident that the acidosis of patients with frank pulmonary oedema need not only be of metabolic but also of respiratory origin. Six of our patients with frank pulmonary oedema had PaCO_2 values exceeding 45 mmHg and none of them had a history of obstructive pulmonary disease (5).

A fall in pH and SB has been described in individual patients with shock (9, 10). The mean pH value in shock patients, however, has been found to be normal (6, 7, 8) and the SB mean value slightly reduced (6, 7). In our study patients with hypotension and shock are dealt with together. Hypotension-shock was more common in patients with low pH values ($p < 0.05$), low SB values ($p < 0.001$) and BE values < -5 mEq/l ($p < 0.001$) as compared to patients with normal and high values. Like patients with frank pulmonary oedema many patients with shock had developed their complication before or on admission when the blood gas sampling was done. Thus it is not possible to deduce from the results of this study whether patients with certain changes in their acid-base balance are more inclined to develop frank pulmonary oedema or hypotension-shock than other groups of patients.

Ventricular as well as supraventricular arrhythmias may be produced in individual patients by metabolic acidosis as well as by respiratory alkalosis induced by mechanical respiration (2, 4). The possibility of an association between ar-

rhythmias and acidosis also appears in the study of Ljungström et al. (8), where pH and SB showed lower values in AMI patients with arrhythmias than in AMI patients with sinus rhythm.

In the present study SVT was more common in patients with low BE and SB values than in those with normal and high values, but had no relation to pH. In our group of AMI patients SVT has been shown to be more common in patients with low PaCO_2 values (5). Thus it seems likely that in many patients with SVT the acidosis is compensated by hyperventilation, which explains the lack of relation between SVT and pH. AF was more common in patients with low SB values but had no relation to pH. Many patients with AF also had low PaCO_2 values (5) and this probably explains the lack of relation between these arrhythmias and pH.

Plicker and Nagle (14) studied acid-base balance in relation toentricular arrhythmias. They found that the incidence of VEB and VT was not related to acid-base disturbances, which is mainly confirmed in this study. VEB in total, or divided into monofocal, multifocal, coupled or R on T showed no relation to pH, SB or BE values and the only relation noted for VT was that there were more patients with VT having low than normal and high BE values. VF however was related to low pH, SB and BE values andentricular asystole was more common in patients with low pH values ($p < 0.001$).

Disorders of A V and intraventricular conduction were related to acid-base balance in the following way. A V I, A V II and CHB did not vary with different values of pH nor with different SB or BE values. LBBB was almost significantly more common in patients with low pH values, significantly more common in patients with BE < -5 mEq/l and highly significantly more common in patients with low SB values than in patients with normal values.

Thus LBBB and VF were related to low pH, SB and BE values and SVT to low SB and BE values. VT was only related to low BE values and asystole to low pH values. High values of pH, SB and BE were not combined with an increased incidence of any arrhythmia. This may be compared to the low hospital mortality rate in alkalemic patients, 8% in patients with SB values > 26 mEq/l or patients with BE values $> +4$ mEq/l.

ACKNOWLEDGEMENTS

This investigation was supported by the Swedish National Association against Heart and Chest Diseases and Carl Yngve Johnson's Foundation, the Swedish Society of Medical Sciences.

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THE EFFECT OF MEFRUSIDE ON PLASMA AND MUSCLE ELECTROLYTES AND BLOOD PRESSURE IN NORMAL SUBJECTS AND IN PATIENTS WITH ESSENTIAL HYPERTENSION

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Abstract. The effect of mefruside on plasma and muscle electrolytes has been studied in 10 normal subjects and in 8 patients with essential hypertension. The normal subjects, 5 men and 5 women, were given 75 mg (to men) or 50 mg (to women) mefruside daily for one week. The hypertensive patients, who were on normal diet, were treated with 25 mg mefruside daily over a period of 5 months. No extra potassium was supplied. Muscle tissue was obtained by needle biopsy from the m. quadriceps femoris before and after the period of mefruside administration. These samples are analysed for water, sodium, potassium, magnesium, chloride and (in the series of normal subjects) total phosphorus. In the normal subjects slight hypochloremic, hypokalaemic alkalosis was recorded in the plasma, in muscle tissue the total water content decreased, as did the potassium content and the K/P ratio, the decreases varying 3-4% of the normal mean value. The blood pressure was not changed. Long-term treatment with mefruside in patients with hypertension resulted in significant blood pressure reduction. Plasma potassium decreased by 0.7 mEq/l; the muscle potassium content was, however, unchanged and the intracellular potassium concentration (calculation based on the chloride method) was significantly increased. The total water content and the extracellular water sodium and chloride content (W) concluded that short-term treatment in normal subjects with high daily dose of mefruside during one week causes slight depletion of intracellular (muscle) potassium, whereas long-term treatment with lower dose in hypertensive patients depletes the extracellular fluid potassium without significantly affecting the intracellular content. The reduction in total water and extra- and intracellular water recorded in the hypertensive patients may have causal relation to the antihypertensive effect.

A preliminary report of this investigation as presented at the Symposium on Diuretics and Hypertension 1 Oct. Norway in 1971 (7, 23).

Benzothiadiazine diuretics are extensively used for basic therapy in the management of patients with essential hypertension. The effect on the blood pressure (BP) is ascribed to reduction of plasma and extracellular fluid volumes by depletion of body sodium and to a direct effect on peripheral vascular resistance, related or unrelated to the depletion of sodium and water (30). Also a number of non-thiazide diuretics, e.g. furosemide, ethacrynic acid, metholazone and spirolactone are known to reduce the BP in hypertensive patients.

A new diuretic, mefruside, has recently been introduced for treatment of arterial hypertension (2, 19, 21, 22). Pharmacological studies indicate that mefruside acts mainly in the ascending loop of Henle (18, 24). The diuretic effect is weaker but more prolonged than that of furosemide and ethacrynic acid which also inhibit sodium reabsorption in this part of the nephron (27). In addition to the increase in sodium excretion, a small or moderate increase in potassium excretion has been observed in acute experiments (2, 12, 31). Similar to thiazides and some other diuretics, mefruside administration may induce hypokalaemia (12, 21, 22).

Short-term experiments in patients with essential hypertension and in normal subjects using a needle muscle biopsy technique have shown that thiazide diuretics and chlorthalidone significantly reduce the intracellular potassium content in muscle tissue, probably in exchange for intracellular sodium (4, 6).

Studies of the effect of long-term treatment

Table I Clinical data for 8 patients with essential hypertension

Patient no.	Sex	Age (yr.)	Fundus hyper-tonicus	Plasma urea (mg/100 ml)	Serum creatinine (mg/100 ml)	Creatinine clearance (ml/min)	Other diagnosis
1	♂	55	II	34	1.3	141	
2	♂	68	I	35	0.8	139	Thrombosis cerebri
3	♀	60	I	29	0.7	66	Neurosis
4	♀	60	I	27	0.6	90	
5	♀	74	II	46	0.95	63	Mb Hashimoto c. myxoedema
6	♂	66	II	49	0.95	108	Infarct. cordis vetus
7	♂	65	I	46	0.8	120	
8	♂	69	I	47	1.05	80	Angina pectoris

with thiazide diuretics on total or exchangeable body potassium have yielded contradictory results (1, 9, 11, 14, 15, 17, 25, 26, 29) some investigators having found a significant reduction, whereas others have found no significant effect.

The purpose of the present investigation was to study

1 the short-term (1 week) effect of mefruside on plasma and muscle electrolytes in normal subjects, and

2 the long-term (5 months) effect of mefruside on BP and plasma and muscle electrolytes in patients with essential hypertension.

MATERIAL AND METHODS

Short-term study on normal subject

Ten healthy volunteers, aged 20-25 years, 5 men and 5 women, were studied. They had no history of cardiovascular or renal disease and were normotensive. Mefruside, 25 mg 3 daily to men and 25 mg 2 daily to women, was administered as tablets for 1 week. Muscle biopsies were performed before and after the period of mefruside administration. At the time of biopsy blood was collected by arterial puncture.

Long-term study in patients with essential hypertension

Eight patients with essential hypertension, aged 60-74 yrs, 5 men and 3 women, were studied. As none of the patients had signs of heart failure, no digitalis glucosides were given. Two patients (nos. 3 and 5) had initially moderate decrease in endogenous creatinine clearance, the others had normal values (>80 ml/min). Renal function, as judged by serum creatinine and urea concentration, and endogenous creatinine clearance were unchanged during the period of investigation. All the patients took normal diet without marked sodium restriction. No extra potassium or antihypertensive drugs except mefruside were administered during the period of investigation. The patients were informed of the purpose of the investigation and gave their voluntary consent. Clinical data are presented in Table I.

The patients were treated with 25 mg mefruside daily as a single morning dose. Before and after 5 months treatment muscle biopsies were performed and venous blood was collected at the time of biopsy care being taken to avoid errors due to sampling and handling of the samples (16).

BP was measured by the same investigator repeatedly during 3 weeks or more before the start of treatment, using the same sphygmomanometer. The pressure was measured after 10 min rest in recumbent position, after 2 min in the standing position, and finally after 10 deep knee-bends. The measurements were repeated monthly during the period of investigation.

Plasma electrolytes, proteins and acid-base parameters were measured by routine methods (3).

Muscle tissue (20-80 mg) was obtained from the m. quadriceps femoris by needle biopsy in the morning after overnight fast. Visible connective tissue was rapidly removed by dissection. The material was divided into 2-4 pieces, weighing 10-20 mg, which were rapidly weighed on an electromagnetic balance. The specimens are dried at 90°C and the water content was obtained by subtraction from the initial weight. Neutral fat was extracted with petroleum ether. The details of the biopsy technique, weighing procedure and fat extraction have been described earlier (2). Dried fat-free specimens obtained before treatment were stored, reweighed and analyzed together with the specimens obtained after the treatment, thus minimizing errors due to changes in equipment, standard solutions, etc. The specimens obtained in the short-term study were analyzed for sodium, potassium, chloride and phosphorus by neutron activation analysis (3) and for potassium and magnesium by atomic absorption spectrophotometry (vide infra). The specimens obtained in the long-term study were treated with 1 N HNO₃, overnight in quartz tubes in order to extract the electrolytes. Sodium, potassium and magnesium contents were determined by atomic absorption spectrophotometry using Technicon AA 4 instrument. Interferences in the atomic absorption spectrophotometry caused by iron and phosphorus were compensated for by adding an excess of these ions to the diluting solutions for standards and samples. The coefficients of variation of the method were as follows: for sodium 1.7%, potassium 1.6% and magnesium 1.8% of the mean normal values.

Chloride was measured by electrometric titration, using

Table II. Body weight, blood pressure, plasma electrolytes, acid-base data and blood glucose in 10 normal subjects before and after 7 days administration of mefruside

	B wt.	BP		Plasma (mEq/l)					PCO ₂ (mmHg)	Standard HCO ₃ (mEq/l)	Blood glucose (mg/100 ml)
		Syst.	Diast.	K	Na	Cl ⁻	Mg ⁺⁺	pH			
Before	61.3	122	78	3.7	137	103	1.89	7.40	40.5	24.6	83.9
After	60.1	120	76	3.1	135	102	1.76	7.42	44.7	26.9	90.6
Difference	-1.2	-2	-2	-0.6	-2	-3	0.07	0.02	4.2	2.3	4.7
Significance	6.00	0.56	0.38	4.69	2.17	2.19	0.54	1.87	2.69	3.11	1.62

Radioactive Thastron ABU 12 with potassium sulphate and silver micro-electrodes. The coefficient of variation of the method was 4.3%.

Tissue water and electrolyte contents were referred to 100 g fat-free solids.

The determination of extra- and intracellular water was based on the chloride method. Chloride is freely diffusible across the skeletal muscle fibre membrane and is distributed according to Nernst's equation (10). Taking the resting membrane potential of muscle in normal man to be 87.2 mV (8), the Cl_i/Cl_e ratio calculated from Nernst's equation will be 26/1; if the total water and chloride content of the muscle tissue and the extracellular concentration of chloride (obtained by correcting the plasma chloride concentration for Donnan factor and factor for plasma water (3)) are known, extra- and intracellular water volumes and intracellular electrolyte concentrations can be calculated (3, 13).

RESULTS

Short-term effect of mefruside in normal subjects (Tables II and III)

The body weight decreased by an average of 1.2 kg as a sign of a reduction in body water. BP was not significantly affected. Plasma potassium and chloride decreased, standard bicarbonate and PCO₂ increased slightly. In muscle tissue

there was a slight fall in potassium content. However the intracellular potassium concentration was not significantly changed.

Long term effects in patients with essential hypertension

Blood pressure A reduction in systolic as well as diastolic and mean BP was observed in all 8 subjects (Table IV). BP measured each month, was decreased during the entire period of investigation.

Plasma electrolytes, plasma protein and acid base balance (Table V). The plasma potassium concentration fell in all patients. The plasma chloride concentration also decreased significantly. The plasma protein concentration increased significantly indicating a slight decrease in plasma volume. Standard bicarbonate and blood pH increased slightly in most of the patients, the difference in blood pH being significant ($p < 0.05$).

Muscle water and electrolytes (Table VI). The total water decreased significantly during the mefruside treatment. Most of this effect could

Table III. Muscle water, electrolytes and glycogen in 10 normal subjects before and after 7 days administration of mefruside

	Per 100 g fat-free solids										Per l H ₂ O _i	
	H ₂ O _{tot} (ml)	H ₂ O _e (ml)	H ₂ O _i (ml)	Cl ⁻ (mEq)	Na (mEq)	K ⁺ (mEq)	K ⁺ (mEq)	Mg ⁺⁺ (mEq)	P (mM)	K/P	Na (mEq)	K (mEq)
Mean												
Before	342	35	287	6.4	88	46.4	46.3	8.9	30.1	1.55	83	161.9
After	342	38	282	6.5	10.4	44.8	44.5	8.8	29.8	1.30	7.8	165.7
Difference	-0.2	2.9	-5.4	0.14	0.79	-1.6	-2.0	-0.08	-0.046	1.5	3.8	3.8
Significance	0.06	0.47	1.39	0.20	1.10	3.14	2.78	1.0	0.69	2.56	1.56	1.41

Activation analysis. Flame photometry.

Table VI. Muscle water and electrolytes before and after 5 months treatment with mefruside 25 mg/day in 8 patients with essential hypertension

Pat. no.		Per 100 g fat-free solids							Per l H ₂ O	
		H ₂ O _m (ml)	H ₂ O _e (ml)	H ₂ O _i (ml)	Cl ⁻ (mEq)	Na (mEq)	K (mEq)	Mg ⁺⁺ (mEq)	Na (mEq)	K (mEq)
1	Before	314	78	306	10.2	14.8	45.5	9.1	13.4	149
	After	334	36	298	5.4	8.0	45.7	8.8	11.9	153
2	Before	342	54	283	7.2	10.8	45.8	8.9	10.5	199
	After	332	67	265	8.7	10.8	45.7	9.2	1.1	172
3	Before	376	72	304	9.6	12.9	46.1	8.3	8.8	151
	After	347	51	297	6.7	8.7	47.7	8.7	5.5	161
4	Before	313	59	334	8.4	11.5	46.4	8.3	9.5	143
	After	334	54	280	7.4	8.5	45.4	9.0	1.6	162
5	Before	364	64	300	8.8	12.5	46.0	9.0	10.2	153
	After	346	67	279	8.7	12.5	43.8	8.2	12.0	156
6	Before	375	84	291	10.8	15.0	43.0	8.5	9.6	148
	After	342	61	281	7.9	10.3	44.9	8.9	6.7	160
7	Before	345	48	297	6.6	9.2	46.1	8.6	7.6	155
	After	331	61	270	7.4	10.7	42.7	8.5	9.2	158
8	Before	345	37	308	5.6	8.0	44.5	8.0	8.7	144
	After	335	49	286	6.6	8.9	44.2	8.2	6.9	155
Mean	Before	364	62	302	8.4	11.8	45.4	8.6	9.8	150
	After	338	56	282	7.3	9.8	45.0	8.7	6.9	160
Difference		-27.0	-6	-20	-1.1	-2.0	-0.4	0.1	-2.9	9.4
t		4.59	0.85	4.67	1.33	1.86	0.68	0.45	2.05	4.88

Significance

tassium content has any adverse clinical effect in this type of patients is, however, far from clear. Evidently the situation is more critical in patients with cardiac insufficiency who are digitalized. Also in connection with marked secondary aldosteronism, e.g. cardiac failure, nephrotic syndrome and liver cirrhosis with ascites, the increased risk of potassium depletion should be considered.

ACKNOWLEDGEMENTS

This study has been supported by grants from the Swedish Medical Research Council (project no B71 19X 1002-07), the Swedish National Association against Heart and Chest Diseases, and Bayer Pharma AB Stockholm, Sweden.

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SENSORY AND MOTOR NERVE CONDUCTION IN THE MEDIAN NERVE IN NORMAL SUBJECTS

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Abstract. The normal nerve conduction in sensory and motor fibres of the median nerve has been studied in 39 females and 28 males, 16-62 years of age. There was no significant sex difference in conduction velocity. The interindividual variation in repeated measurements was 0.9-1.8 m/sec. The conduction velocity decreased with age in all segments, and the interindividual variation was 3.4 to 4.5 m/sec (S_{95}). Sensory fibres conducted faster than motor fibres of the same segment, but the difference became gradually effaced with age. The sensory conduction velocity in the proximal segment (wrist-elbow) decreased more rapidly with age than in the distal segment (dign-wrist). Fast and more slowly conducting fibres are almost equally affected by age resulting in an increase in the temporal dispersion of the sensory action potential. The amplitude of the sensory potential (G_{50} , μ V) was a function of the temporal dispersion and of the distance of the nerve electrode from the nerve. With these corrections the amplitude was independent of age, i.e. there was no evidence of reduction in the number of fibres. The sensory threshold to electrical stimuli increased with age and—independent of age—with the slowing of the sensory conduction velocity between wrist and elbow.

During the past 20 years the determination of sensory and motor conduction velocity has obtained a prominent position in the study of the peripheral nerve function. When measuring the conduction velocity with a standard technique and procedure, age is probably the most important source of variation in normal subjects. Motor and sensory fibres, however, do not seem to be affected in parallel. Thus data presented by La Frazia and Canestrari (17) indicate that the conduction in mixed nerves becomes relatively more slowed by age than in motor fibres. This is consistent with findings by Buchthal and Rosenfalck (3), who examined purely sensory and motor fibres. They showed that sensory nerve conduction was faster than motor in young per-

sons, while in old persons (70-88 years) this relationship was in fact reversed. Other electrophysiological parameters change with age e.g. a significant decrease in the amplitude of the sensory nerve action potential has been demonstrated (3, 15). The present study however shows that this was solely due to an increase in the temporal dispersion of the action potential.

This study was designed to derive clinically applicable limits of the normal inter and intra individual variation of sensory and motor conduction parameters considering the effect of age and the interaction between the recorded parameters. The relative slowing of conduction in sensory and motor fibres and in distal and proximal segments was analysed.

MATERIAL AND METHODS

The material comprised 20 females and 28 males, 16 to 62 years of age. The age distribution was the same in the two sexes. Clinical symptoms or signs of peripheral nerve dysfunction were absent and there was no evidence of diseases known to predispose to peripheral neuropathy. Four persons were examined at weekly intervals, four times each, in order to calculate the intrasubject variation.

Electrophysiological methods

The sensory and motor conduction velocity in the median nerve was measured as described by Buchthal and Rosenfalck (3). Sensory fibres were stimulated through ring electrodes placed distally on the 1st and 3rd digits. The stimulus strength was 57 ± 15 mA (mean \pm S.E.). Action potentials were recorded unipolarly at wrist and elbow through needle electrodes,

which were adjusted until the lowest stimulus strength was reached that could just evoke an action potential in the abductor pollicis brevis muscle (motor threshold, T_m). T_m was 0.7 ± 0.03 mA (wrist) and 0.5 ± 0.03

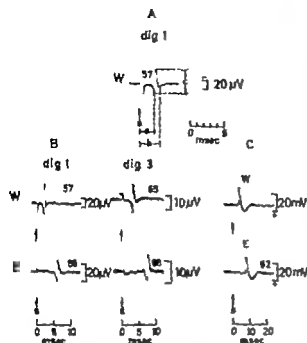


Fig. 1. Sensory and motor action potentials in the median nerve.

(A) Sensory action potential recorded at the wrist (stim. dig. 1); (\sim stimulus = 60 mA); and b = latency (msec) in 1st and 2nd positive peak respectively; $b-a$ = temporal dispersion, c = peak-to-peak amplitude (μ V).

(B) Sensory action potentials recorded at the wrist (W) and the elbow (E) evoked by stimulation of digits 1 and 3 (60 mA).

(C) Muscle action potentials (m. abd. pol. brev.) following stimulation of the median nerve at wrist and elbow.

Figures above the potentials indicate the conduction velocity (m/sec). Temperature near the nerve = 35°C.

mA (elbow) Action potentials were displayed on 3-channel electrocytograph (DNA Copenhagen). The time base was 0.5 or 0.25 msec/mm. Motor fibres were stimulated through the electrodes at wrist and elbow. The stimulus strength was 9.0 ± 0.4 mA. Action potentials were recorded by concentric needle electrodes placed in the end-plate zone of the abd. pol. brev. muscle. The time base was 0.5 and 1.0 msec/mm.

The interelectrode distance (ID) was the mean of at least three measurements, assuming straight course of the nerve between the electrodes (fingers stretched). ID averaged 111 ± 0.7 mm (wrist-thorax), 130 ± 1.3 mm (digit 1-wrist), 145 ± 1.5 mm (digit 3-wrist), and 254 ± 2.8 mm (wrist-elbow). The extremity was heated prior to and throughout the examination. The skin temperature on the palm of the fingers and on the thenar eminence was $35.0 \pm 0.1^\circ\text{C}$. Temperatures at wrist and elbow measured near the nerve with thermopile averaged $36.0 \pm 0.1^\circ\text{C}$.

Nerve conduction parameters (Fig. 1). In sensory fibres the latency was measured to the nearest 0.1 msec

from the onset of the stimulus to the first positive peak of the triphasic action potential. At the wrist the latency was also measured to the second positive peak. The temporal dispersion of the action potentials was expressed as the difference between the latencies to the two peaks. The amplitude was measured peak-to-peak to the nearest 0.1 μ V. In motor fibres the latency was measured from the stimulus onset to the initial deflection from the baseline of the muscle action potential. The peak-to-peak amplitude was measured to the nearest 0.1 mV. The conduction velocity (m/sec) was equal to the interelectrode distance divided by the difference in latencies.

The sensory threshold to electrical stimuli, I (mA), was the weakest impulse that could be discerned by random stimulation.

Statistical procedures

All statistical analyses were conducted in accordance with conventional procedures (4). The cumulative frequency distribution of the data plotted on probability paper showed that the best fit to normal (Gaussian) distribution as obtained by logarithmic transformation (\log_{10}) of the amplitude of action potentials (Fig. 2) and of the sensory threshold to electrical stimuli. Other variables fitted in with a normal distribution on a linear scale.

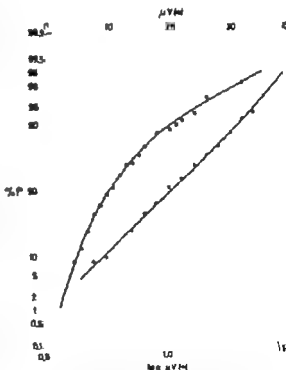


Fig. 2. The cumulative frequency distribution plotted on probability paper of the amplitude of sensory nerve action potentials recorded at the wrist after stimulation of digit 3 ($N=60$). O = amplitudes in μ V ● = amplitudes after transformation into \log_{10} space.

Table I. Average sensory and motor conduction velocity (m/sec) in segments of the median nerve (normal persons, 16-62 years)

	Females (n=20)		Males (n=28)		Total (n=48)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Age (y)	41.6	12.6	36.2	13.0	38.4	13.0
Conduction velocity						
Digit 1-wrist						
1st peak	52.3	4.6	52.9	5.4	52.6	5.0
2nd peak	35.8	2.6	37.5	4.9	36.7	4.3
Digit 3-wrist						
1st peak	57.4	3.8	59.1	4.4	58.4	4.2
2nd peak	39.8	3.1	41.7	4.4	40.8	4.1
Wrist-elbow (sensory)	65.7	3.7	65.5	5.4	65.6	4.8
Elbow-erast (motor)	62.2	3.7	61.9	4.6	62.0	4.2

There is no significant difference between females and males ($p = 0.10$).

RESULTS

Conduction time and conduction velocity (Table I)

The distal motor latency (DML) was 3.2 ± 0.1 msec (S.D. 0.5). In 33 double determinations (two recording electrodes) the mean difference was 0.2 msec (range 0.0-0.9). The average intraindividual variation was 0.2 msec. DML increased with the

length of the interelectrode distance (wrist-abd. polli. brev. muscle), $r = 0.41$, $p < 0.001$. However the variation in DML was only slightly reduced when corrected to the mean interelectrode distance (63 mm) according to the equation (13):

$$\text{corrected latency} = \text{observed latency} - \left(\frac{63 - \text{ID}}{1} \right)$$

Table II. Intraindividual variation (δ - m/sec) of sensory and motor conduction velocity in the median nerve (four normal persons, examined at weekly intervals four times each)

	Subject no.				δ^a	Range
	1 (16 y.)	2 (17 y.)	3 (18 y.)	4 (32 y.)		
Digit 1-wrist	53.0	56.2	57.0	58.8	± 1.3	53-59
	53.6	56.4	52.0	61.4		
	55.2	55.0	54.2	60.4		
	53.3	56.2	55.4	59.1		
Digit 3-wrist	61.7	61.6	65.2	65.7	± 1.8	61.2-71.1
	62.3	62.5	58.1	65.1		
	61.2	61.0	59.4	62.4		
	61.0	62.7	61.1	64.1		
Wrist-elbow	70.1	71.9	68.3	65.9	± 0.9	65.4-76
	68.5	72.8	68.5	65.4		
	67.5	70.5	68.2	64.4		
	68.5	72.2	66.8	64.8		
Elbow-erast	63.3	61.3	64.3	62.1	± 1.6	61.1-65.2
	60.9	61.7	64.4	63.1		
	61.4	62.7	63.5	60.0		
	65.2	64.6	65.0	61.1		

$$\delta = \sqrt{\frac{\sum_{i=1}^N (x_i - \bar{x})^2}{(\sum_{i=1}^N N_i) - Kc}}$$

x - single observation, \bar{x} - mean of observations within the single person, Kc - no. of persons, N - no. of observations in each person. The denominator denotes the number of degrees of freedom.

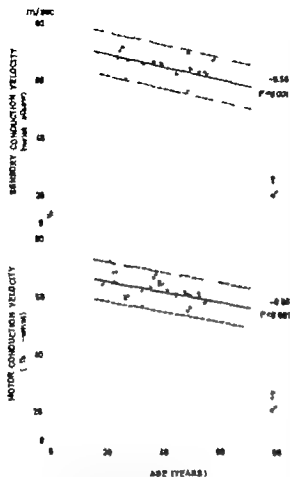


Fig. 3 Sensory and motor conduction velocities in the median nerve (wrist-elbow) related to age in 48 normal persons. --- regression line, ---- ± 2 standard error of estimate (S_{est}).

(ID = interelectrode distance (mm), V_m = motor conduction velocity elbow-wrist (m/sec)). The corrected DML, on average 31 ± 0.1 msec (S.D. 0.4) did not increase with advancing age $p > 0.05$.

There was no systematic variation between the sensory and motor conduction velocity and the temperature of the segment (range 34.0–37.7 °C) or the stimulus strength. In 33 double determinations of the motor conduction velocity the difference ranged from 0.0 to 5.3 m/sec, on average 1.2 m/sec (S.D. 1.3). The intraindividual variation in sensory and motor conduction velocity amounted to 0.9–1.8 m/sec (Table II). This represents 19–43 % of the standard deviation of the mean values in the total material, or 1.4–3.1 % of the average conduction velocities. The temperatures differed less than 2.0 °C in the same

person. The average conduction velocities in males and females did not differ significantly $p > 0.10$ (Table I). A significant slowing of the conduction velocity was recorded with advancing age in all segments and in fast and more slowly conducting sensory fibres (Fig. 3, Table III). This correction reduced the range of normal variation by 10–20 %.

Considering all persons irrespective of age the conduction velocity in sensory fibres between wrist and elbow was faster than in motor fibres, on average 4.0 m/sec (S.D. 3.0), $p < 0.001$ (paired observations). The difference, ranging from -1.3 to $+10.6$ m/sec, became eliminated with age, $r = -0.34$, $p < 0.01$. The conduction velocity in sensory fibres from digit 3 was on average 7.3 m/sec (S.D. 4.1) faster in the proximal (wrist-elbow) than in the distal (digit 3-wrist) segment, $p < 0.001$. This was not due to differences in temperature. The difference in conduction velocity decreased with age, $p < 0.05$. There was no systematic difference between the sensory conduction velocities in fibres from digits 1 and 3 in the proximal segment, $p > 0.8$ while in the distal segment the sensory conduction velocity was on average 5.8 m/sec faster in fibres from digit 3 $p < 0.001$. This is at least partly due to the fact that the true nerve length between digit 1 and wrist is systematically underestimated by percutaneous measurements due to the obtuse angular course of that segment.

Table III. Relationship between sensory and motor conduction velocity (median nerve) and age: Y (msec) = $a + bX$ (years) (48 normal persons, 16–62 years)

	$Y = a + bX$		S_{est}	r	P
	a	b			
Digit 1-wrist					
1st peak	59.0	-0.16	4.3	-0.43	=
2nd peak	44.8	-0.20	3.2	-0.64	
Digit 3-wrist					
1st peak	64.8	-0.17	3.5	-0.52	=
2nd peak	49.0	0.21	3.0	-0.67	
Wrist-elbow (sensory)	73.9	-0.22	3.8	-0.79	
Elbow-wrist (motor)	69.0	-0.18	3.4	-0.56	

Standard error of estimate:
 < 0.01 < 0.001

Table IV Amplitudes of sensory action potentials (median nerve) and muscle action potentials (m. abd. polli. brv.) (normal persons, 16-62 years)

Recording	Site of stimulation	Amplitude (log ₁₀ values)			δ ^b	Normal variation (recalculated from log ₁₀ scores)	
		Mean	S.E.	S.D.		Mean	95 range
<i>Sensory action potentials, μV (n = 60)^a</i>							
Wrist	Digit 1	1.4820 ± 0.0271		0.2118	0.0577	30.4	11.4-80.5
	Digit 3	1.0589 ± 0.0260		0.2034	0.0548	11.4	4.5-29.2
Elbow	Digit 1	1.0282 ± 0.0241		0.1868	0.0555	10.7	4.5-25.2
	Digit 3	0.8329 ± 0.0225		0.1746	0.0512	6.8	3.0-15.2
<i>Muscle action potentials, mV (n = 93)^a</i>							
M. abd. polli. brv.	Wrist	1.2650 ± 0.0152		0.1476	0.1251	18.4	9.3-36.3
	Elbow	1.2162 ± 0.0164		0.1587	0.1333	16.4	7.9-34.2

^aFour persons were examined four times each.

^bIntraindividual variation.

The action potential was recorded from two sites in 33 subjects.

The muscle action potential

The amplitude of the muscle action potentials showed considerable inter and intraindividual variation (Table IV). In 33 double determinations the amplitudes showed differences ranging from 0 to 22 mV. The amplitudes were not correlated with age. Two per cent of potentials were poly phasic, displaying more than four phases.

The sensory nerve action potential

The temporal dispersion of action potentials recorded at the wrist following stimulation of digits 1 and 3 averaged 1.1 ± 0.03 msec (S.D. 0.2) and 1.4 ± 0.04 msec (S.D. 0.3). Due to an almost equal slowing of the conduction velocity in fast and more slowly conducting fibres the temporal dispersion increased significantly with age.

The total variation in the amplitude of sensory action potentials appears from Table IV. The intraindividual variation constituted 27-30% of the total variation. The amplitude (log₁₀ μV) was a function of the temporal dispersion, $r = -0.73$ and -0.58 , $p < 0.001$ (digits 1 and 3 Fig. 4). The amplitude (a) decreased with advancing age (b). This, however, was due to the joint correlation with the temporal dispersion (c). Thus in a multiple correlation analysis between (a), (b) and (c) the partial correlation coefficient for age was far from significant, $r_{ab} = -0.05$ and 0.04 (digits 1 and 3). In pathological action potentials and in potentials recorded at the elbow it was not always

possible to measure the temporal dispersion accurately with the present technique. However this variable could be replaced by the latency to the first positive peak at the expense of about 5-10% of the explained variation in amplitude (Table V). There was no systematic variation with the strength of stimuli used, but the amplitude was inversely correlated with the motor threshold $r = -0.47$ and -0.48 $p < 0.001$ (digits 1 and 3). This was independent of other sources of variation.

The sensory threshold to electrical stimuli

T for digits 1 and 3 averaged 3.5 mA (95% range = 2.0-6.1 mA) and 3.1 mA (95% range = 1.7-5.4 mA). T was systematically higher in digit 1 than in digit 3 $p < 0.001$. T (log mA) increased with age, $r = 0.64$ and 0.71 $p < 0.001$ (digits 1 and 3), and with slowing of the sensory conduction velocity between wrist and elbow $r = -0.66$ and -0.67 $p < 0.001$ (Fig. 5). As the age and the conduction velocity were also significantly correlated, $r = -0.66$, the multiple correlation between T (a), age (b) and V (c) was analysed (digit 3 48 normal persons). The two latter variables added an independent contribution to the amount of explained variation in T , $r_{ab} = -0.48$, $p < 0.001$ and $r_{ac} = -0.37$ $p < 0.02$, and more than 50% of the total variation in T could be referred to covariation with these variables, $R_{abc} = 0.76$, $p < 0.001$.

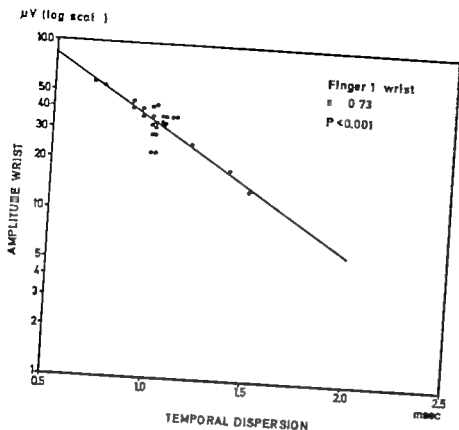


Fig. 4a.

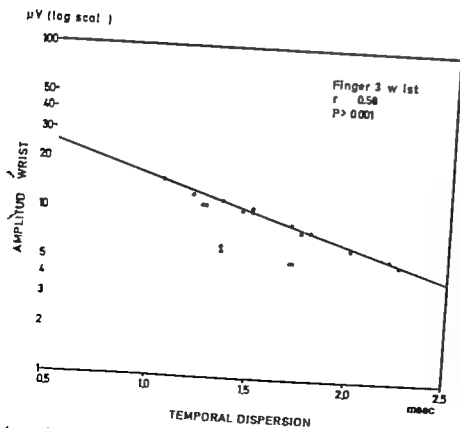


Fig. 4b.

Fig. 4 The amplitude of the sensory action potential (log scale) recorded at the wrist as a function of the temporal dispersion of the potential. The regression equations are: (a) (digit 1), $Y(\log \mu V) = 2.31 - 0.77X$ (msec), $S_{yx} = 0.14$, and (b) (digit 3), $Y(\log \mu V) = 1.60 - 0.39X$ (msec), $S_{yx} = 0.17$.

Table V The amplitude ($Y = \log \mu V$) of the sensory action potential (median nerve) as a function of the latency ($X = \text{msec}$) to the first positive peak (normal persons, 16-62 years)

Recording	Site of stimulation	$Y = -bX$		S_{yx}	r
			b		
Wrist	Digt 1	2.7979	-0.5371	0.1467	-0.72
	Digt 3	2.0241	-0.5081	0.1788	-0.47
Elbow	Digt 1	2.1369	-0.1768	0.1566	-0.54
	Digt 3	1.9695	-0.1633	0.1412	-0.58

Standard error of estimate.
 *** 0.001.

DISCUSSION

Technical problems associated with the neurographic procedure used in this study have been discussed elsewhere (3-9). The present results were not influenced by variations in temperature within the range 34-37.7 C nor by variations in the

stimulus strength. An exponential correlation was present between the amplitude of the sensory action potential and the motor threshold, which was used to indicate the distance of the near nerve electrode from the nerve. This is in keeping with data presented by Trojaborg and Sindrup (28) (radial nerve), and compatible with theoretical (19) and experimental (3) findings, indicating that an electrode position very close to the nerve e.g. $T_m < 0.5 \text{ mA}$, becomes particularly critical for the potential amplitude.

The conduction velocity in different types of fibres and segments of the median nerve decreased linearly with age, and the degree of variations in this material was of the same order as in other reports (3, 5, 6, 15, 18, 22, 29). A significant sex difference, as suggested in previous reports (15, 18), could not be demonstrated in the present study.

The error of analysis in double determinations of the motor conduction velocity averaged 1.2 m/sec. This is considerably less than observed by Gassel (7), while compatible with the experimental error of 5-7% calculated by Trojaborg (26), who compared the conduction velocity in the right and left arm. In the present study the intra-individual variation ranged from ± 0.9 to $\pm 1.8 \text{ m/sec}$ in different nerve segments, indicating that 95% of repeated observations may be expected to deviate less than $\pm 4 \text{ m/sec}$ in each direction. This amounts to 19-43% of the total interindividual variation, which implies that in longitudinal studies the conduction velocity may theoretically deteriorate 2-5 times the intrasubject variation and still be within the 95% limits of variation in the total group of normal persons. Thus a suspected nerve dysfunction cannot be excluded by

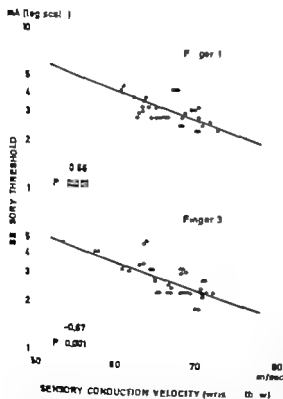


Fig. 5 The sensory threshold to electrical stimuli (log scale) for digits 1 and 3 as function of sensory conduction velocity between wrist and elbow. The regression equations are: (digit 1), $Y (\log \text{ mA}) = 1.73 - 0.02X$ (m/sec), $r = -0.86$ and (digit 3), $Y (\log \text{ mA}) = 1.62 - 0.02X$ (m/sec), $r = -0.87$.

a single value within the lower end of normal variation. These figures are in good agreement with those given by Henriksen, 1.9 m/sec (10), and Trojaborg, 1.4 m/sec (27) while Honet et al. (14) obtained standard deviations of 4–6 m/sec for differences in conduction velocity in different nerves measured at intervals greater than one week. They used surface electrodes, which may have increased the inaccuracy in measuring the interelectrode distance, and the temperature was not controlled.

It has been shown that the conduction in purely motor nerve fibres is slower than the conduction of afferent impulses in mixed nerve fibres (16, 17, 20, 21, 22, 25) as well as in purely sensory fibres of the same segment (3). The present results are consistent with these findings and furthermore confirm the trend, previously mentioned (3, 17), towards a gradual elimination of the difference with advancing age $p < 0.01$. This study also suggests that the conduction in proximal segments becomes more severely affected by age than in distal, as evidenced by the significant decrease of the difference in conduction velocity between distal and proximal segments of sensory fibres and also by the absence of a significant increase of the distal motor latency within the age range, 16–62 years. So far there is no obvious explanation of this phenomenon.

According to reconstructions made by Buchthal and Rosenfalck (3) the normal triphasic action potential recorded at the wrist originates from myelinated fibres, 6–12 μ in diameter. The first positive peak originates from fast conducting fibres of about 11 μ . The second positive peak of the compound action potential, however, is more complex, comprising the second positive peak of fast conducting fibres, but also the first positive peak of more slowly conducting fibres of about 7 μ diameter. The ageing process equally affected the fast and slow component, as also indicated by a significant increase in the temporal dispersion of the compound action potential (3). As shown by the multiple correlation analysis this fully accounted for the reduction in potential amplitude with age. Thus, slowing of the nerve conduction with age was not accompanied by electrophysiological evidence in favour of a reduction in the number of active fibres.

The exponential increase of the threshold for electrical shocks with age follows the general pat-

tern for sensory stimuli (11). Hinchcliffe (12) suggested that the pattern of ageing might be attributed to degenerative changes in the cerebral cortex, referring to the linear reduction of the brain weight (1, 23) and of the cerebral cortex cell count (2). Degenerative changes in the receptor organs were also suggested. The present study shows that the temporal dispersion of the sensory action potential increases with a decrease in the rate of impulse propagation in afferent nerves. This was accompanied by a rise in the sensory threshold independent of age. The fact that the conduction velocity was measured after stimuli of at least ten times the threshold does not invalidate the correlation, since it has been shown that the action potentials following subthreshold, threshold, and supramaximal stimuli are propagated with the same speed (3, 30). Recently Rosenfalck and Buchthal (24) showed that the amplitude of the action potential at threshold stimuli was the same in normal subjects as in patients with elevated sensory threshold, whereas the temporal dispersion was three times greater. Their conclusion was in agreement with that of Gilliat and Wilton (8), that the higher threshold stimuli might serve to activate more fibres, hereby compensating for the drop in the amplitude of the threshold action potential due to the greater temporal dispersion by slowed conduction velocity.

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THE⁷PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

V. Sensory and Motor Conduction Velocity

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Abstract. Sensory and motor nerve conduction has been measured in 56 patients with chronic renal failure. Slowing of nerve conduction was present in one or more segments in 34 of 39 patients with a 24-hour creatinine clearance below 10 ml/min/1.73 m². The impairment involved upper and lower extremities, motor and sensory fibres, distal and proximal segments, and fast and more slowly conducting fibres. The amplitude of sensory action potentials was reduced, mainly due to increased temporal dispersion and to increased incidence of irregularities in the shape of potentials. The electromyographic contraction pattern at maximal effort in the b.d. polli. brev. muscle was rarely abnormal, whereas the pattern in the ext. dig. brev. muscle was compatible with moderate to severe loss of motor units in 21 of 39 patients. All patients with electrophysiological signs of impaired nerve function had slowed motor conduction in the common peroneal nerve and/or slowed sensory conduction in the median nerve. The demonstration in the present material of an almost uniform slowing of nerve conduction in all segments contrasted with the reported distribution of structural changes, predominantly located in the distal parts of the legs. The hypothesis is put forward that the slowing of nerve conduction is not solely dependent on structural changes, but also on a universal toxic effect upon the nerve axon membrane by uraemic toxin(s) other than urea and creatinine.

Several authors have demonstrated impaired motor nerve conduction in the median, ulnar, radial, common peroneal, and posterior tibial nerves in patients with chronic renal failure (CRF) (5, 12, 16-19, 21, 24, 36, 38). Sensory fibres are affected with equal frequency (18) and even more severely (5, 19), and the affection comprises distal as well as more proximal segments of the median nerve (27). Previous reports concerned the fastest conducting fibres, but a preliminary study suggested that slower conducting fibres are affected as well (27).

The aim of this study was: 1) To establish the extent of impaired nerve conduction in patients with conservatively treated CRF in particular the relationship between the conduction in the upper and lower extremities, in motor and sensory fibres, distal and proximal segments, and fast and more slowly conducting fibres. 2) To estimate the diagnostic significance of electrophysiological parameters and to establish their value in longitudinal studies.

The generalized slowing of the nerve conduction was considered incompatible with the pattern of structural changes in the nerve, which is predominantly localized to the distal part of the legs. However it has been shown that uraemic serum inhibits the active extrusion of intracellular sodium, resulting in a reduction in the transmembrane resting potential. It is suggested that this may be an important pathophysiological background of slowed nerve conduction in uraemic patients.

MATERIAL AND METHODS

Normal controls. The results of sensory and motor nerve conduction in the median nerve in 48 normal persons (20 females, 28 males, 16-62 years of age), have been reported (32). The motor nerve conduction in the common peroneal nerve as measured by members of the staff in this laboratory in 41 normal persons, 15-65 years of age. Normal values appear from the text.

Patients. Fifty-six patients with CRF were randomly selected from a larger patient material (29). Their ages ranged from 15 to 61 years (25 females) and from 17 to 59 years (31 males), the age and sex distribution matching closely with that in normal controls. None of the patients were on haemodialysis program at the time of the examination. The kidney function was expressed by the 4-hour endogenous creatinine clearance,

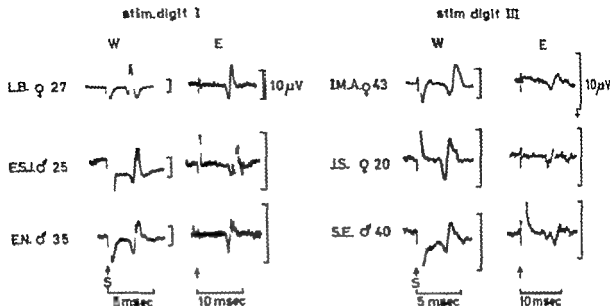


Fig. 1 Sensory action potentials recorded at wrist and elbow in patients with CRF.

C_{cr} , ml/min), which was corrected to a standard BSA of 1.73 m^2 among other reasons because females and males are pooled together. C_{cr} ranged from $1.38 \text{ ml/min/1.73 m}^2$ being below $10 \text{ ml/39 patients}$ (normal range $78\text{--}110 \text{ ml/min/1.73 m}^2$ (14)). Clinical signs of neuropathy were present in 29 patients. According to criteria previously presented (30), the neuropathy was graded as mild in 13 patients and moderate to severe in 16. Four of the 27 patients without objective neurological findings complained of sensory symptoms suggestive of neuropathy.

Electrophysiological examination. Some patients were examined more than once during the course of pretransplant renal failure. The results reported here refer to the last examination prior to the institution of regular hemodialysis programme or renal transplantation.

The sensory and motor conduction in the median nerve was measured as described previously (32). The motor threshold averaged $0.8 \pm 0.03 \text{ mA}$ at wrist and $0.7 \pm 0.03 \text{ mA}$ at elbow (mean \pm S.E.). The stimulus used to evoke motor or sensory responses was about 10 times the motor or sensory threshold. The temperatures varied $34.8 \pm 0.2^\circ\text{C}$ (digits I and 3) and $35.6 \pm 0.1^\circ\text{C}$ (wrist and elbow). These values are comparable with those in the control material (32). In addition to parameters defined previously (32) irregularities in the shape of the sensory action potential were studied. These consisted of notches which broke the continuity without changing the direction of the positive and negative deflection, and of separate peaks when the triphasic course as interrupted by deflections in the opposite direction (Fig. 1). Irregularities or located in the initial phase is the positive deflection from the base line to the first positive peak, the intermediate phase between the first and second positive peaks, and the terminal phase from the second positive peak until

stable base line was reached. Late peaks were often difficult to distinguish from noise, especially when recorded at the elbow.

Motor fibres of the common peroneal nerve at stimulated through needle electrodes placed near the nerve at the capitulum fibulae and the ankle and muscle action potentials are recorded by concentric needle electrodes placed in the end plate zone of the extensor dig. brevis muscle. The temperature of cap. fib. and ankle averaged $34.1 \pm 0.2^\circ\text{C}$.

The electromyographic pattern at maximal voluntary effort recorded in the bductor polli. brevis and the extensor dig. brevis muscles, as compared in 29 patients. The normal response had an amplitude of 1.5 mV or more. The pattern was described according to Simpson (17) as 1) interference pattern, 2) reduced interference pattern and 3) discrete activity.

RESULTS

Motor nerve conduction

Common peroneal nerve. In one of 49 patients supramaximal stimulation failed to elicit a muscle response. The mean conduction velocity in the 43 patients averaged 41 m/sec compared to 50 m/sec in normal persons ($p < 0.001$) (Table 1). The distal motor latency (ankle - ext. dig. brevis) was increased in only 11 patients (23%), whereas the motor conduction velocity (cap. fib.-ankle) was reduced in 30 patients (62%) (Fig. 1). The amplitude of the evoked muscle potential was reduced in 9 patients ($< 3.5 \text{ mV}$), but the average

Table 1. Average conduction velocities (m/sec) in normal persons (15-62 years), and in patients with chronic renal failure (15-61 years)

Segment	Controls		Patients	
	Mean	S.D.	Mean	S.D.
Median nerve				
Digit 1-wrist				
1st peak	48	52.6	50	45.9
2nd peak	48	36.7	43	30.6
Digit 3-wrist				
1st peak	48	58.4	42	51.1
2nd peak	48	40.8	41	35.2
Wrist-elbow (sensory)	48	65.6	48	56.9
Elbow-wrist (motor)	48	62.0	42	55.2
Proximal nerve				
Cap. Elb-axilla	41	50.0	33	41.1

amplitude of all patients (11.8 mV) did not differ significantly from the normal. The potentials were polyphasic in 13 patients.

Median nerve The distribution of distal motor latencies (wrist-abd. polli. brevis) was the same in patients with CRF (3.2 msec, S.D. 0.5) as in normal controls (3.2 msec, S.D. 0.5), whereas the motor conduction velocity (elbow-wrist) was significantly slowed in 32 patients (58%) (Table 1, Fig. 4). The amplitude of the evoked muscle potential averaged 19.0 mV (range 6-42 mV), i.e. as in normal controls. The potentials were polyphasic in 9 of 55 patients as compared to 1 of 48 normal controls, $p < 0.05$.

Electromyographic pattern at maximal effort

Only 4 of 29 patients showed a reduced interference pattern or discrete activity indicating a moderate to severe loss of motor units in the abd. polli. brevis muscle, compared to 21 patients in the ext. dig. brev. muscle $\chi^2 = 18.0$ $p < 0.001$. However the amplitude was rarely significantly reduced (Fig. 3). There was no correlation between the contraction pattern at maximal effort and the motor conduction velocity. Thus an interference pattern was found in more than half the patients with slowed nerve conduction (Table II). Seven of 11 patients without clinical signs of neuropathy showed loss of motor units. There was no significant difference between the creatinine clearance



Fig. 2. Relationship between the distal motor latency and the motor conduction velocity in the common peroneal nerve in 48 patients with CRF. \square - 95% range of normal variation.

in patients with normal and pathological patterns ($p > 0.2$).

Sensory nerve conduction

The average sensory conduction velocity (median nerve) was reduced by 13-17% of the normal value in distal and proximal segments as well as in fast and slow conducting fibres ($p < 0.001$) (Table I). Figs. 4, 5 and 6 show the uniform incidence and degree of slowing in different seg-



Fig. 3. Amplitude and shape of the electromyographic pattern at maximal effort in 29 patients with CRF. IP - interference pattern, MP - mixed pattern - reduced interference pattern (17), SO - single oscillation pattern - discrete pattern (12). The amplitude is normally above 1.5 mV.

Table II Electromyographic pattern at maximal effort (ext. dig. brev. muscle) in 29 patients with chronic renal failure related to motor conduction (V_m , common peroneal nerve), clinical neuropathy and kidney function ($CCr = ml/min/1.73 \text{ m}^2$)

EMG pattern	No. of pts.	V_m (m/sec)		Clinical neuropathy		CCr	
		>43	<43	Absent	Present	Mean	Range
Interference	8	3	5	6	2	10.3	1.2-34.1
Reduced interference or discrete activity	21	7	14	7	14	5.2	1.0-37.5

ments and groups of fibres. The mean ratios between conduction velocities in distal and proximal segments and in slow and fast fibres in patients with CRF were identical with those in normal subjects with comparable age and sex distribution. The sensory conduction velocity however was slower than the motor conduction velocity in the wrist-elbow segments in 18 patients (33%) as compared to 4 normal subjects (8%) $\chi^2 = 7.68, p < 0.01$.

The sensory action potential (Fig. 1). The temporal dispersion of action potentials recorded at the wrist was significantly increased.

Irregular potentials were rarely recorded at the

wrist following stimulation of digit 1 whereas irregularities occurred twice as frequently in patients as in normal controls when stimulating digit 3 (Table III). The incidence of irregular potentials at the elbow was 20-30% higher in patients with CRF than in normal controls. Separate peaks occurred twice as frequently and desynchronization was more widespread. Irregularities were most pronounced in patients with severe renal failure.

The average amplitude of action potentials was significantly reduced in patients with CRF. There was no systematic variation with the motor threshold, which indicated the distance of the new nerve electrode from the nerve. However when

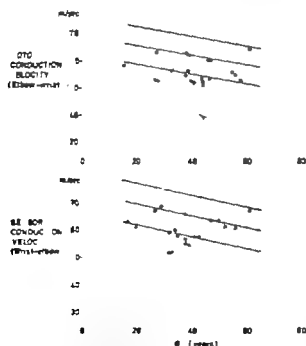


Fig. 4 Motor (upper) and sensory (lower) conduction velocity in the median nerve related to age in patients with CRF. The lines indicate the mean value and 95% range of normal variation corrected for age.

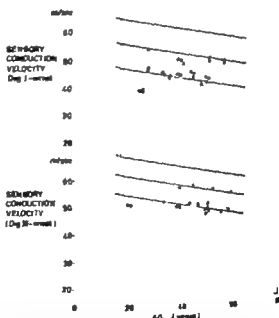


Fig. 5 Sensory conduction velocity in distal segments of the median nerve related to age in patients with CRF. The conduction velocity was calculated from the latency to the first positive peak of the triphasic action potential. The lines indicate the mean value and the 95% range of normal variation corrected for age.

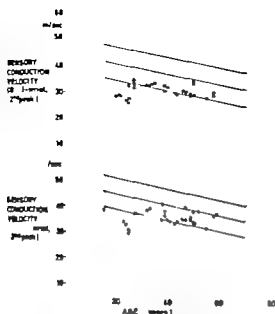


Fig. 6 Sensory conduction velocity in distal segments of the median nerve related to age in patients with CRF. The conduction velocity was calculated from the latency to the second positive peak of the triphasic action potential. The lines indicate the mean value and the 95% range of normal variation corrected for age.

plotted against the temporal dispersion of the potentials recorded at the wrist, most amplitudes fitted in with the expected range of normal variation (Fig. 7). The temporal dispersion of potentials recorded at the elbow could not be measured accurately but in normal subjects (32) the amplitude was significantly correlated with the latency to 1st positive peak ($r = -0.54$, $p < 0.001$).

Table III. Irregularities in sensory action potentials recorded at the wrist and elbow in normal persons and patients with chronic renal failure

Recording	Site (deg)	No. of pts.	Irregular potentials		Notches only (n)	Separate peaks (n)	Initial phase (°)	Inter-mediate phase (n)	Terminal phase (n)
			(n)	(%)					
Normal	I	48	3	6	3	0	0	3	8
	III	48	7	13	7	0	0	7	1
Elbow	I	48	24	50	14	10	2	20	9
	III	48	31	67	16	18	8	27	14
Patients									
Wrist	I	56	4	7	3	1	1	4	2
	III	52	17	33	12	5	3	11	10
Elbow	I	56	43	77	21	22	11	37	20
	III	41	35	85	8	27	9	33	18

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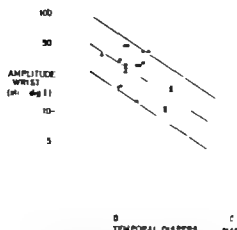


Fig. 7 The amplitude (log scale) plotted against the temporal dispersion of sensory action potentials recorded at the wrist in patients with CRF. The lines indicate the regression line and the 95% range of variation in normal persons.

Fig. 8 shows the plot in patients with CRF. More potentials had amplitudes below the lower limit of expected variation. This coincided with a greater degree of desynchronization.

The sensory threshold to electrical stimuli (digit 1 or 3) was significantly elevated in eight patients, in all of whom the peripheral nerve function was clearly impaired as judged from other electrophysiological or clinical parameters.

Covariation of impaired nerve conduction

The diagnostic significance of other electrophysiological parameters than motor and sensory con-

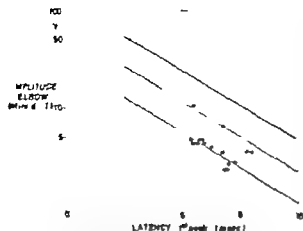


Fig. 8 The amplitude (log scale) plotted against the latency to the first positive peak of sensory action potentials recorded at the elbow in patients with CRF. The lines indicate the regression line and the 95% range of normal variation in normal persons.

duction velocities was negligible. Impaired nerve conduction was observed in one or more segments in 47 patients (84%) and none of the remaining 9 showed other electrophysiological evidence of neuromuscular affection.

The covariation of impaired nerve conduction

in the four segments examined was analysed in 48 patients. As illustrated in Fig. 9 there was a considerable overlap. The prevalence of impaired nerve conduction varied from 0.52 (median nerve, digit-wrist) to 0.62 (common peroneal nerve, cap. fib-ankle) and the diagnostic sensitivity of a single segment did not exceed 0.50. This indicates that the prediction of normality from a normal value in a single segment alone would be incorrect in at least half the patients. In order to discover impaired nerve conduction in all affected patients, the diagnostic programme should comprise determination of the motor conduction in the common peroneal nerve and of the sensory conduction in distal and proximal segments of the median nerve.

DISCUSSION

Diagnostic significance

The covariance and interdependence among variables of the peripheral nerve function have earlier been pointed out (30-31, 35). The present neurographical study provided further data on the same topic. However from a diagnostic point of view the amount of information does not grow infinitely with the application of more variables. Thus, notwithstanding their descriptive neurophysiological interest, most parameters discussed in this paper were diagnostically redundant.

The motor conduction in the common peroneal nerve was the most valuable single diagnostic parameter but all the diagnostic information available from the present programme was only obtained when the examination was extended to comprise also the sensory conduction velocity in distal and proximal segments of the median nerve. A thorough electromyographic examination may reveal signs of a neurogenic affection prior to slowing of nerve conduction, the presence of fibrillation potentials being particularly sensitive as an indicator (40). On the other hand, in 11 patients with CRF Liberson et al. (43) found fibrillation in only 10 of 19 patients with slowed nerve conduction. Furthermore, as a parameter for longitudinal studies of the peripheral nerve function the conduction velocity is more suitable than electromyography.

Extent of impaired nerve conduction

A significant slowing of nerve conduction was found in one or more segments in 38 of 39 pa-

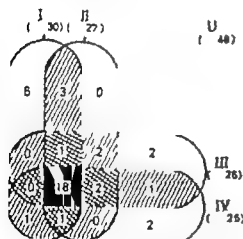


Fig. 9 Venn diagram illustrating the covariation in 48 patients with CRF. (I) between I - motor conduction elbow-peroneal nerve, II - motor conduction elbow-median nerve, III - sensory conduction elbow-median nerve, wrist-elbow segment and IV - sensory conduction elbow-median nerve, digit-wrist segment. Figures outside the circle indicate total number of patients. No showed conduction velocities below the 95% limit of normal variation in the single segments. Figures within the circle indicate number of patients who showed impaired nerve conduction in 1, 2, 3 or 4 of the segments.

patients with severe CRF ($C_{Cr} < 10 \text{ ml/min/1.73 m}^2$) irrespective of clinical signs of neuropathy. Moreover in "unaffected" nerve segments the recorded conduction velocities were often low borderline values. This applies to upper and lower extremities, motor and sensory fibres, and distal and proximal segments, in keeping with previous observations (16, 18, 19, 27). Thus a generalized slowing of the nerve conduction constitutes an integral part of the uremic syndrome.

The motor conduction in the common peroneal nerve was relatively more affected than that of the median nerve. In the wrist-elbow segment of the median nerve the V_s/V_m ratio was below 1.0 more frequently than seen in normal subjects. Distal and proximal segments of sensory fibres of the median nerve were affected to the same degree contrary to findings in diabetic patients, in whom the affection was more severe in the distal segments (22). Preliminary studies (27) also showed a significant slowing of the sensory conduction between elbow and axilla in patients with CRF which was not seen in diabetic patients. These differences may suggest a different genesis of slowed nerve conduction in the two diseases.

In normal persons it was shown that the conduction velocity of fast and more slowly conducting fibres, as indicated by the latency to the first and second positive peak of the triphasic sensory action potential, deteriorated almost in parallel with advancing age (32). The study presented here showed that in patients with CRF the conduction velocity of the two components was significantly reduced, with equal frequency and to about the same degree (87–83% of the normal mean value), as was originally suggested from the observation of a significant increase in duration of the sensory action potentials (27). Reduced nerve conduction in slow conducting fibres has also been demonstrated in diabetic patients (22) and in patients with polyneuropathy of different etiologies (11).

Number of active fibres

In the individual subject the sensory action potential amplitude varies with the number of activated fibres when recorded under identical circumstances (10). The present study showed that changes in the temporal dispersion as well as in regularities in the shape of the potentials should

be considered before an estimate of the number of active fibres is made from the potential amplitude. Thus, at the wrist, changes in the amplitude could be regarded as a function of the temporal dispersion alone while in potentials recorded at the elbow the higher incidence and severity of irregularities were probably responsible for the relatively greater reduction in amplitude in patients with CRF than in normal controls. These findings are consistent with the analysis of the sensory action potential in patients with different types of polyneuropathy by Buchthal and Rosenfalck (11), who found evidence of block of fibres only in severely affected patients.

The electromyographic pattern at maximal effort suggested a moderate to severe loss of motor units (9) in the ext. dig. brevis muscle in 1 of the 29 patients examined, but rarely so in the abductor pol. brevis muscle in the same group of patients, consistent with findings by Sod et al (38) in 11 patients with CRF. Loss of motor units combined with normal motor conduction velocity can be accounted for by assuming a diffuse axonal degeneration sparing some of the fastest conducting fibres. On the other hand, normal number of functioning motor units is not incompatible with slowing of conduction in motor nerves.

In conclusion, electrophysiological evidence of a significant reduction in the number of nerve fibres was a frequent finding in the common peroneal nerve, but rarely seen in the median nerve.

Pathophysiology of slowed nerve conduction in CRF

In most reports on the peripheral nerve function in various diseases the morphological basis of slowed nerve conduction is assumed to be a combination of axonal degeneration and segmental demyelination. This is mainly based on the experimental evidence that severe segmental demyelination is accompanied by slowed nerve conduction (25, 26), the presumed pathophysiological mechanism being that demyelination results in decrease in the ohmic resistance of the myelin sheath (39), so reducing the current density at the nodes of Ranvier. This in turn delays the depolarization of the membrane potential at the adjacent nodes, hence delaying the propagation of

the impulse (26). Extensive demyelination eventually may result in a conduction block.

According to this hypothesis neurophysiological changes in CRF as observed in this material would imply the presence of morphological changes in nerves of lower and upper extremities and in distal and proximal segments. They should occur in patients with moderate and terminal renal failure and in patients with and without clinical evidence of neuropathy. Demyelination in single fibres should be widespread, since it is well known from the effect of local anesthetics that the saltatory impulse volley can readily jump over one or more completely blocked internodes without any appreciable reduction in conduction velocity (6). Thomas (41) finds it questionable whether the early stages of a demyelinating process can account for the mild slowing of the nerve conduction often observed. Axonal degeneration has been suggested instead. This abnormality however is predominantly a distal phenomenon.

Histopathological studies so far have been confined to patients in terminal renal failure, and the reported findings do not fulfil the above mentioned implications. In the upper extremity demyelination and axonal degeneration was stated to be absent or considerably less pronounced than in the legs (1-15). In the lower extremity autopsies showed sparing of more proximal nerve segments while in several patients distal segments (e.g. sural nerve biopsies) displayed a combination of axonal degeneration and segmental demyelination (42). For these reasons an alternative or at least a supplementary explanation is desirable.

During the past decade a growing amount of evidence has been reported in favour of a biochemically induced inhibition of the transmembrane sodium pump in patients with CRF.

1) Bolfe et al. (7) showed that the average resting membrane potential in forearm muscles was reduced by about 25% in patients with CRF. Cunningham et al. (13) obtained similar results in the anterior tibial muscle. The reduction of the membrane potential was accompanied by a significant rise in the intracellular concentration of sodium.

2) Welt et al. (43) demonstrated an abnormally high intracellular sodium concentration in red blood cells in uremic patients. The activity of the Na-K-activated ATPase was diminished in red cell ghosts, while the concentration of ATP was

superabundant. Plasma from uremic patients with a high red cell sodium concentration reduced the activity of Na-K-activated ATPase prepared from normal red cell ghosts. This effect disappeared following chronic hemodialysis.

Bergström and Hultman (2) failed to demonstrate high intracellular sodium concentration in muscle biopsies from uremic patients. Their calculations from the Nernst equation were based on the assumption of a normal resting membrane potential.

3) Blittar showed that the sodium efflux from the crab muscle fibres (*Astale*) (3) and from the oocytes of the common toad (*Bufo-bufo*) (4) was markedly inhibited when uremic plasma was added to the medium. This was not so with dialyzed plasma or with normal plasma.

4) Klahr et al. (20) and Bricker et al. (8) demonstrated that a low molecular fraction of uremic serum, obtained by Sephadex G 25 gel filtration, effectively inhibited the transepithelial sodium transport through isolated frog skin, accompanied by a fall of the transepithelial potential difference. An inhibition of the sodium efflux from erythrocytes was also observed. The same fraction inhibited the sodium-dependent PAH uptake in rat kidney slices, but this effect was diminished after hemodialysis and in one case disappeared completely 2-3 weeks after renal transplantation.

To summarize, low molecular humoral factor(s) in uremic serum inhibit the Na-K-activated ATPase, resulting in a defective transmembrane sodium pump, an inhibition of the sodium efflux, and a reduction of the resting membrane potential. As suggested by Bricker et al. (8) this may affect the functional integrity of multiple organs and organ systems.

The general slowing of the nerve conduction in patients with CRF could be explained by the above mentioned findings. Irrespective of morphological changes the above mentioned sequence of events resulting in a reduced resting membrane potential would reduce the current density at the nodes of Ranvier slowing the depolarization of the membrane and hence the rate of impulse propagation. The degree of inhibition should be considered as a function of advancing uremic intoxication, and as being reversible following renal transplantation. This is in keeping with the close correlation between the conduction velocity and

the kidney function (33) and with the biphasic recovery of nerve conduction following renal transplantation (28) where the initial rapid phase may coincide with the disappearance of the trans-epithelial transport inhibiting factor in serum (8).

Indirect experimental evidence in favour of this model was obtained when nerves in normal persons were subjected to gradual depolarization during short-lasting ischemia (30 min) (34). The nerve conduction was gradually slowed to about 70% of the pre-ischemic value, i.e. of the same order as observed in terminal renal failure (33). The affection comprised slow and fast conducting fibres as well as distal and proximal segments of the nerve. Changes in the degree of myelination of nerve fibres distal to the compression appear highly improbable during the short lasting period of ischemia.

In conclusion, slowed nerve conduction may reflect demyelination and axonal degeneration when present. In patients with CRF the generalized affection of impulse propagation in peripheral nerves, however, does not fit in with the pattern of histopathological abnormalities. It is therefore suggested that the slowing of nerve conduction is also due to an inhibition of the transmembrane sodium pump mediated by humoral toxic factor(s) in serum, resulting in a reduction of the current density at the nodes of Ranvier. The degree of inhibition and thus of slowing of nerve conduction is dependent on the severity of renal failure. It is reversible following recovery of renal function.

ACKNOWLEDGEMENTS

This study is supported by grants from "The Elton Foundation", "København i Odense, Joben og Hanne Weinmann, Jøft Beudorff's legat" and "Olfredsen af Skovsbo Frederik Laurids Pedersen legat til støtte for lægevidenskabelig forskning".

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

VI The Relationship between Sensory and Motor Nerve Conduction and Kidney Function, Anemia, Age, Sex, and Clinical Neuropathy

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Abstract. The sensory and motor conduction velocity in the median and common peroneal nerve in 46 patients with chronic renal failure (CRF) showed a linear correlation with the endogenous creatinine clearance (C_{cr}) in a multipathic system. The nerve conduction became impaired more severely and earlier in the course of CRF in males than in females. A significant reduction of the conduction velocity can be expected in half of the patients when the kidney function is reduced to about 10% of normal, and in terminal renal failure only few patients will show conduction velocities within normal limits. When the covariation with C_{cr} was taken into account, the conduction velocity was not influenced by the serum concentrations of creatinine and urea, indicating that neither of these represent the neurotoxic substances in the uremic syndrome. There was no difference between the conduction velocities in patients with and without clinical signs of neuropathy when correction was made for incomparability in the degree of renal failure. Furthermore there was striking disparity between the predominance of clinical neurological signs in the distal parts of the legs and the almost uniform slowing of the nerve conduction in upper and lower extremities. These two latter findings indicate that the correlation between clinical findings and nerve conduction is more complicated than might be assumed from previous studies.

A generalized slowing of the peripheral nerve conduction was shown to be an integral part of the uremic syndrome in advanced chronic renal failure (CRF) (15). The aim of this study was to describe the correlation with the kidney function expressed by the glomerular filtration rate for creatinine. The conduction velocity might be influenced by the degree of anemia (2, 9-11), which does not always parallel the glomerular filtration rate of the kidney and by the age and

sex of patients. It was the second object of this report to reconsider these problems. Though the relationship between clinical neuropathy and the degree of slowing of the nerve conduction previously reported (7, 8, 12) has been evaluated in the light of the covariation with the kidney function.

MATERIAL AND METHODS

The material was the same as described previously (15), where details were given about the kidney function, expressed by the 24-hour endogenous creatinine clearance (C_{cr} , ml/min) corrected to body surface area of 1.73 m² about age and sex distribution, and about the incidence and degree of clinical neuropathy. The degree of anemia was expressed by the serum concentrations of creatinine (range 4-32.5 mg/l) and urea (range 0.50-3.90 g/l). Normal values in this laboratory were: creatinine 7-13 mg/l and urea 0.20-0.45 g/l. All laboratory analyses were made at the time of the neurophysiological examination.

This study concerns the motor and sensory conduction velocity (m/sec) in the fast conducting fibres of the common peroneal and the median nerve. Other electrophysiological parameters have been described previously (15).

Statistical analysis. In an analysis of the cumulative percentage frequency distribution the best fit to normal (Gaussian) distribution was obtained by logarithmic transformation (\log_{10}) of the following variables: creatinine clearance (\log ml/min/1.73 m²), serum urea (\log g/l) and serum creatinine (\log mg/l) concentrations. The relationship between conduction velocity (v) and kidney function (C_{cr}) (4) was tested by regression analysis. When other numerical variables showed significant correlation with the conduction velocity multiple correlation analysis (4) was applied, testing whether a new variable (c) increased the amount of

Table I. Correlation between (Y) sensory and motor conduction velocity (m/sec) and (X) kidney function ($C_{Cr} = \log \text{ml/min/1.73 m}^2$) in chronic renal failure

ΔV = age-adjusted deviation from the normal mean value

Segment	No. of pts.	$Y = a + bX$			
		b	S_{EY}	r^2	
<i>Median nerve</i>					
Distal 1-wrist	56	41.9	5.2	3.9	0.52
ΔV	56	-12.5	7.1	3.9	0.63
Distal 3-wrist	52	47.5	4.3	3.5	0.49
ΔV	52	-12.5	6.7	3.5	0.63
Wrist-elbow	56	52.2	6.3	5.3	0.47
ΔV	56	-15.4	9.1	5.4	0.60
Elbow-wrist	55	49.6	7.4	4.4	0.60
ΔV	55	-14.6	9.6	4.6	0.69
<i>Peroneal nerve</i>					
Cap fib-ankle	48	34.2	9.0	4.7	0.68

S_{EY} Standard error of estimate.

r^2 Correlation coefficient, $p < 0.001$ in all segments.

explained variation in the conduction velocity independently of the kidney function, i.e. whether the coefficient of partial determination, r^2_{res} , differed significantly from zero. Analysing differences in the conduction velocity between two groups of patients, it was first tested whether the groups were comparable with

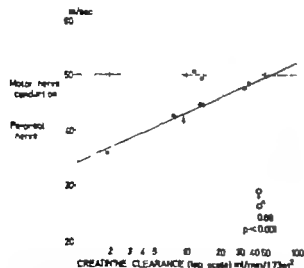


Fig 1 The relation between motor conduction velocity in the common peroneal nerve and kidney function. Shaded area = 95% range of normal variation. The arrow indicates the creatinine clearance level at which 50% of patients can be expected to show pathological values. The regression equation appears from Table I. The distribution of observations around the regression line in males and females differed significantly ($p < 0.02$) see Fig. 4.

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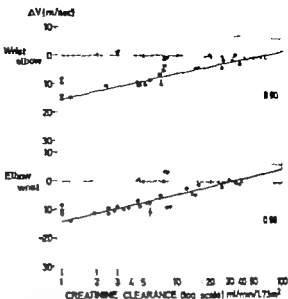


Fig 2 Sensory and motor conduction velocity (wrist-elbow median nerve) related to the kidney function. The observations were expressed as the deviation (ΔV m/sec) from the age-adjusted mean value in normal persons. Shaded area = 95% range of normal variation, (± 2 times the standard error of estimate (S_{EY})) for the regression, conduction velocity on age, in 48 normal persons, 16 to 62 years of age (14). Arrows indicate the creatinine clearance level at which 50% of patients can be expected to show pathological values. The regression equations appear from Table I.

respect to age and kidney function. If a difference was observed, e.g. in the kidney function, Wilcoxon rank sum test (6) was applied, testing whether the conduction velocities in the two groups showed a different distribution around the common regression line relating conduction velocity and kidney function (C_{Cr}).

RESULTS

Kidney function and azotemia

There was a highly significant correlation between the conduction velocity (m/sec) and the kidney function ($\log C_{Cr}$) in all nerve segments (Table I Figs. 1 and 2). In the median nerve the correlation coefficient was considerably improved when the degree of slowing of nerve conduction was adjusted for age, expressed by the deviation, $\Delta V \pm \text{m/sec}$ (V = velocity), from the normal mean value for the age of the individual patient (Fig. 2).

Statistically a significant reduction of the conduction velocity can be expected in half the patients or more when the kidney function becomes

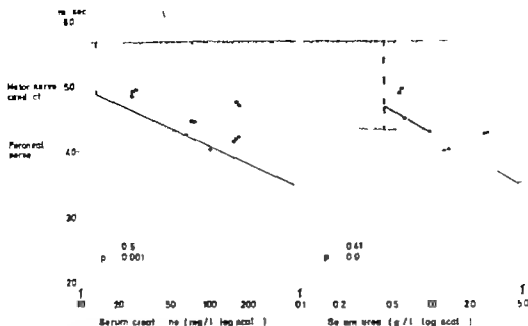


Fig. 3 Motor conduction velocity (common peroneal nerve) as function of the creatinine and urea concentrations in serum (log scale). Shaded area—95% range of normal variation. Vertical stippled lines—upper limit

normal level of serum creatinine and serum urea. A analysis of the covariation with the creatinine clearance is summarized in Table II.

reduced below the creatinine clearance level at which the regression line intersects with the lower limit of normal variation (arrows in Figs. 1 and 2). For the motor nerve conduction in the common peroneal nerve the intersection occurred at C_{Cr} 9.6 ml/min/1.73 m² i.e. corresponding to a residual kidney function of about 10% of the normal. A similar degree of affection of the sensory and motor conduction in the median nerve (wrist-elbow segment) was reached at a somewhat later stage in the course of renal failure, C_{Cr} 7.4 and 5.8 ml/min/1.73 m² respectively. In terminal renal failure (C_{Cr} 1 ml/min/1.73 m²) the nerve conduction had decreased to an average of 12–16 m/sec below the normal mean value, and the scatter around the regression line (S_{res} in Table I) indicated that a significant reduction can be expected in almost all patients.

The conduction velocity was inversely correlated with the degree of azotemia (Fig. 3). Table II shows a multiple correlation matrix, relating () motor conduction velocity in the common peroneal nerve (V_m), (b) creatinine clearance (C_{Cr}), (c) serum urea, and (d) serum creatinine. Considering the covariation with the creatinine clearance the partial correlation coefficients for urea

and creatinine were far from significant. On the other hand, even when the effect of the actual degree of azotemia was taken into account, it was still possible to explain 27% of the residual variation in the conduction velocity by variations in the kidney function (C_{Cr}) as $r_{ab.cd} = 0.52$, $p < 0.001$. This was also indicated by the multiple correlation coefficient, $R_{a.bcd}$, which was identical with the simple correlation coefficient between

Table II. Multiple correlation between () motor conduction velocity (V_m m/sec), peroneal nerve (b) creatinine clearance (C_{Cr} log ml/min/1.73 m²), (c) serum urea (log g/l) and (d) serum creatinine (log mg/l) in 48 patients with chronic renal failure

Correlation coefficients (r_{xy})

	(b) C_{Cr}	(c) Serum urea	(d) Serum creatinine
(a) V_m	0.6773	-0.4066	-0.5137
(b) C_{Cr}		-0.6395	-0.8370
(c) Serum urea			0.7336

Partial correlation coefficients $r_{ab.cd} = -0.52$
 $r_{cd.ab} = -0.52$

Multiple correlation coefficient

Table III Age (γ), kidney function (C_{Cr} , $\log \text{ ml/min}/1.73 \text{ m}^2$) and nerve conduction (m/sec) in female and male patients with chronic renal failure

	Females			Males			p
	N	Mean	S.D.	N	Mean	S.D.	
Age	25	40.7	12.3	31	36.5	11.5	>0.10
C_{Cr}	25	0.89	0.44	31	0.66	0.45	<0.05
<i>Median nerve</i>							
Digit 1-wrist	25	47.6	4.2	31	44.4	4.4	<0.05
Digit 3-wrist	24	52.6	3.6	28	49.8	4.0	<0.05
Wrist-elbow (sensory)	25	59.1	5.8	31	55.5	5.3	<0.05
Elbow-wrist (motor)	25	56.7	6.0	30	53.9	5.0	$0.10 > p > 0.05$
<i>Peroneal nerve</i>							
Cap. fib-ankle	23	44.0	4.7	25	38.5	5.8	<0.005

motor conduction velocity and creatinine clearance, $r = 0.47$.

Age and sex

The nerve conduction was more severely impaired in young than in old patients (Figs. 4-5, 6 in the preceding paper (15)). However a multiple correlation analysis showed that this was due to the fact that the kidney function was generally more reduced in the young than in the old patients, $r = 0.47$, $p < 0.001$.

The peripheral nerve conduction was more severely affected in male than in female patients

(Table III). As the average creatinine clearance was also lower in males than in females ($p < 0.05$), a Wilcoxon rank sum test was applied, testing the distribution of observations in the two groups around the common regression lines given in Table I. In the common peroneal nerve the distributions differed significantly ($p < 0.02$, Fig. 1). In the median nerve a similar tendency was seen, although not statistically significant at the 5% level ($0.10 > p > 0.05$). Accordingly Fig. 4 illustrates that the nerve conduction tended to be more affected less severely and at a later stage in the course of progressive renal failure in females than in males.

Clinical neuropathy

The average conduction velocities were significantly slowed in patients both with and without clinical signs of neuropathy. This also applied to sensory and motor fibres, and to distal and proximal nerve segments in the upper extremity where objective clinical findings were rarely observed. One or more nerve segments were affected in 19 of 27 patients without clinical neuropathy and in 28 of 29 patients with clinical signs of neuropathy. The impairment was confined to a single segment in 10 patients, 5 of whom had clinical signs of neuropathy (Fig. 5).

The average conduction velocity was significantly lower in patients with neuropathy than in those without (Table IV). There was a uniform age and sex distribution, but the kidney function was more affected in patients with clinical neuropathy, $p < 0.005$. However a Wilcoxon rank sum

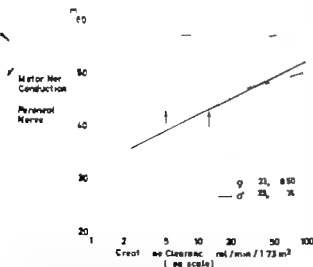


Fig. 4 The regression of the motor conduction velocity (common peroneal nerve) on the kidney function, calculated for females ($Y = 5.4X + 39.2$) and male patients ($Y = 9.9X + 32.0$) separately. Single values are shown in Fig. 1.

Clinical neuropathy	Segment	No. of pts	%	Cum %
Absent	Finger 1-Wrist	27	eeee	18
	Finger 3-Wrist	28	e/e eeeee	34
	Wrist-Elbow	27	ee ee eee eee	37
	Elbow-Wrist	27	ee eeee ee ee	41
	Cap hb -Ankle	23	ee/ ee/ /ee eee	44
Present	Finger 1-Wrist	29	eeeeeeeeeeee	48
	Finger 3-Wrist	28	eeeeeeeeee// eeeeeee	69
	Wrist-Elbow	28	eeeeeeee eee eeeeeeeee	72
	Elbow-Wrist	28	eeeeee/eeeeeeeeee e	75
	Cap hb -Ankle	26	eeeeee/eeee/eeee eeeee	81

e reduced values

/ not measured

Fig. 3 Frequency and distribution of impaired nerve conduction in segments of the median and common peroneal nerves in individual patients with and without clinical signs of neuropathy. Empty boxes indicate that the conduction velocity was within the 95% of normal range. Each vertical column in the % groups represents one patient.

test failed to demonstrate any difference between the distribution of observations in the two groups around the common regression line correlating conduction velocity and kidney function (C_{Cr}), $p > 0.10$. Hence, unlike the above mentioned sex difference, there was no relationship between the presence or absence of clinical neuropathy and the degree of impaired nerve conduction which could not be explained by the variation in kidney function. Fig. 6 illustrates the extent of overlapping.

DISCUSSION

Kidney function and acetonuria

Lilothelm (11) showed that slowing of the motor conduction (ulnar nerve) was significantly correlated with the non-protein-nitrogen concentra-

tion in serum. In individual patients with chronic renal failure Jepsen et al (9) demonstrated that an increase in the serum creatinine concentration was accompanied by a reduction of the conduction velocity. Blagg et al (2) found that the correlation between the conduction velocity and the creatinine concentration was superior to that with urea. They inferred that this was probably "because serum creatinine levels reflected renal function more accurately".

This concept was confirmed in the present investigation, in which the kidney function was determined directly. Slowing of the nerve conduction was a linear function of the creatinine clearance in a semilogarithmic system, in keeping with previous observations concerning the impairment of vibratory perception (13). When the kidney function became reduced to about 10% of the

Table IV Age (y), kidney function (C_{Cr} , log ml/min/1.73 m²), and nerve conduction (m/sec) in patients with chronic renal failure with and without clinical neuropathy

	Without clinical neuropathy			With clinical neuropathy			p
	N	Mean	S.D.	N	Mean	S.D.	
Age	27	40.2	12.0	29	36.6	11.8	0.10
C_{Cr}	27	0.94	0.42	29	0.60	0.43	<0.005
Median nerve							
Digit 1-wrist	27	47.8	11.8	29	44.1	4.5	<0.005
Digit 3-wrist	28	52.4	3.5	26	49.8	4.2	<0.05
Wrist-elbow (sensory)	27	59.4	5.1	29	54.6	6.1	<0.005
Elbow-wrist (motor)	27	56.6	5.7	28	53.7	5.2	<0.05
Peroneal nerve							
Cap. fib.-ankle	23	43.3	5.7	25	39.2	6.4	<0.05

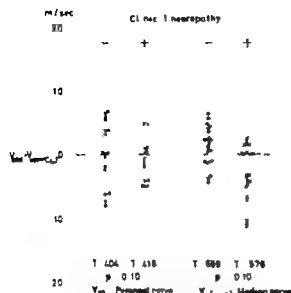


Fig. 6. The distribution of conduction velocities around the regression line relating conduction velocity and kidney function ($\log C_{cr}$) in patients with and without clinical neuropathy. Each point in the columns represents the difference (\pm m/sec) between the observed value and the conduction velocity calculated for the creatinine clearance of the patient according to the regression equations given in Table 1. T-values = the Wilcoxon rank sum in each group.

normal, slowing of nerve conduction could be expected in half the patients. The common peroneal nerve became affected slightly earlier than the median nerve but the slowing of nerve conduction followed a nearly parallel course in comparable segments of the two nerves. Thus a decrease in C_{cr} from 10 to 1 ml/min/1.73 m² resulted in a reduction of the conduction velocity by about 9 m/sec. In terminal renal failure conduction velocities within the normal range can be expected in only a few patients, and since the intrasubject variation amounts to 20–40% of the normal range (14), low borderline values may in fact represent a significant slowing of the nerve conduction in the individual patient. This was directly indicated by the improvement of the nerve conduction which could be observed following renal transplantation in such patients (16). These findings further support the concept of a general impairment of the impulse propagation as a function of progressive renal failure.

The serum concentrations of creatinine and urea are both rough estimates of the kidney function, as they are influenced by the lean body mass

(creatinine) and the dietary protein intake (urea). Neither creatinine nor urea appears to display any toxic action on the peripheral nerve function, at least not in clinically relevant concentrations (1). This is consistent with the results of the multiple correlation analysis in this study which failed to demonstrate any correlation with the conduction velocity independent of the creatinine clearance. The neurotoxic substance(s) linking reduced kidney function and impaired nerve conduction are as yet unknown. This is probably only part of the general question of the connection between chronic renal insufficiency and the uraemic syndrome. The criteria that any agent(s) show a toxic action on peripheral nerves should be that the correlation coefficient (or possibly the multiple correlation coefficient) for the relationship between the concentration of the agent and the conduction velocity is equal to or higher than that obtained for the total kidney function, e.g. C_{cr} .

Age and sex

With comparable severity of renal failure the degree of slowing of the nerve conduction was uninfluenced by age. This differs from the general picture of clinical neuropathy (17), and in particular the vibratory perception threshold (13), which showed that males in the upper age classes were more liable to become affected. The fact that chronic renal failure was more pronounced in young patients may reflect an unforeseen primary selection of the material. Thus it is conceivable that patients had been submitted to a selection prior to admission to the renal unit, considering the more favourable possibilities of applying hemodialysis and/or renal transplantation in young patients at the time of the present investigation (1966–69).

Previous reports in this series (13, 17) demonstrated that clinical signs of neuropathy and impaired vibratory perception were less frequent and less severe in female than in male patients, in conformity with other studies (20, 21, 22). The present investigation further indicated a significant sex difference in the degree of slowing of the motor conduction in the common peroneal nerve, and the same tendency was suggested in the median nerve. This is at variance with observations by Dobbstein et al. (5), who found no

significant difference between the motor conduction velocity (ulnar and posterior tibial nerves) in males and females. Unfortunately their patient groups were matched with respect to the serum creatinine concentration. However the actual serum creatinine concentration is greatly influenced by the lean body mass, which is generally less in females than in males. Hence equality in the average serum creatinine levels in fact indicates a greater reduction of the average kidney function in female than in male patients. Consequently it remains possible that the slightly higher average motor conduction velocity in females in their material might reach a statistically significant level if correlated with the kidney function corrected to a standard body surface area. For the time being there is no obvious explanation of the observed sex difference.

Clinical neuropathy

Chaumont et al. (3) first showed that the conduction velocity was significantly reduced in uremic patients without clinical evidence of neuropathy. This was soon confirmed by Prewick and Jeremy (18). "Subclinical neuropathy" is also well known in other types of neuropathy (19). It has been shown that the nerve conduction is more reduced in uremic patients with clinical neuropathy than in those without (7, 8, 12). However although patient groups have been carefully matched with respect to age and sex, due attention has apparently not been paid to differences in the degree of renal failure.

The present study showed a striking discrepancy between clinical findings and peripheral nerve conduction. This concerns (a) the incidence, (b) the severity and (c) the pattern of distribution of clinical and neurophysiological findings.

(a) Slowed nerve conduction was present in two thirds of patients without clinical evidence of neuropathy. This was not a fortuitous finding, as it was demonstrable in two or more nerve segments in 14 of the 19 patients affected. In comparison, slowing was confined to one segment in five patients with clinical neuropathy. There was apparently no critical conduction level below which clinical signs invariably became manifest. Thus marked slowing of the nerve conduction was compatible with absence of symptoms and with completely normal clinical findings, while

on the other hand young patients might show unequivocal clinical evidence of neuropathy although the conduction velocity was only reduced to values which would be considered normal in clinically unaffected subjects of higher age.

(b) If slowing of the nerve conduction were more pronounced in patients with clinical neuropathy than in those without, it should be so in respect of the degree of renal failure. This was not the case. Both groups of patients in the present study showed a uniform distribution of the conduction velocities around the common regression line relating conduction velocity and creatinine clearance. Hence differences between mean values in the two groups could be referred to in comparability with respect to the degree of renal failure. A difference between patients with and without neuropathy has also been reported in other diseases. Thus, in diabetic patients, Lamontagne and Buchthal (10) showed that the severity of clinical findings was significantly correlated with an arbitrary grading of the degree of impaired motor nerve conduction (common peroneal nerve) which also included the distal motor latency and the amplitude and shape of the evoked muscle action potential. As judged from the scatter of their observations, several patients could be expected to show clinical neuropathy without abnormal electrophysiological findings. For obvious reasons a correction for the severity of the primary disease was not possible.

(c) The difference in the pattern of affection is perhaps the most illustrative dissociation between clinical findings and peripheral nerve conduction. The nerve conduction was impaired with equal frequency in the lower and upper extremity in motor and sensory fibres, and in distal and proximal segments of sensory fibres (15). The minor differences in the degree of affection could not account for the entirely different pattern of clinical findings, which were predominantly sensory and in most patients confined to the distal part of the lower extremity.

Thus, although clinical neurological signs must somehow be related to the ability to conduct sensory and motor impulses, the correlation with the conduction velocity is apparently far from simple. Longitudinal studies of the development and course of clinical and neurophysiological parameters of peripheral neuropathy may elucidate this problem in more detail.

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IRON FORTIFIED BREAD

A Long-term Controlled Therapeutic Community-based Experiments with Ferrous Sulphate-enriched Flour

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Abstract. The hematologic effect of ferrous sulphate enriched flour has been evaluated in a long-term, community-based experiment. During a period of 20 consecutive months 17 000 metric tons of iron-fortified flour were produced at Tena Mill in Stavanger, Norway (total iron content 6.2 mg/100 g flour). There were neither complaints of rancidity of the flour nor of any harmful effect on its baking qualities. The monitoring of the effect of the iron-fortification programme was performed on an experimental group of 207 women of child-bearing age living in Stavanger. A control group of 15 women is established in Bergen. Blood examinations (Hb, Hct and MCHC) are performed at the start of the experiment and reported on three occasions during the study. The results failed to give conclusive evidence of the hematologic effect of the iron supplement. However, the results obtained in the few cases with the lowest circulating Hb levels (Hb ≤ 12 g/100 ml) suggested that the added iron was absorbed and utilized for Hb synthesis. The authors have recommended to the Norwegian health authorities that all white flour should be fortified with ferrous sulphate. It seems reasonable to apply the same level of enrichment as was recently proposed by the US Commissioner of Food and Drug Administration (total level of iron 5 mg/100 g flour).

Food fortification is a public health measure aimed at improving and maintaining the health of individuals in the population through the provision of adequate levels of nutrient intake (13). Iron is one of the nutrients which requires special consideration.

Based on normal values for Hb concentration and other hematological parameters, we have in previous studies estimated the prevalence of anemia in various population groups (18). Iron-deficiency anemia is rather prevalent in Norway. It is not uncommon in schoolchildren and is frequently found in women of reproductive age and in the elderly.

At present the level of iron in the average Norwegian diet is only about 4-5 mg/1000 kcal (28, 29). Infants and small children, elderly people, patients and physically inactive individuals with a low caloric intake as well as those with an increased iron requirement due to growth, convalescence, menstrual bleeding, pregnancy and other special needs, are likely to receive an insufficient supply of iron. The recommended daily intake for women of reproductive age is, for example, 18 mg iron, which should be provided in no more than 1850-400 kcal (4). Thus the low-calorie consumer (<2000 kcal/day) requires a diet with no less than 10 mg iron/1000 kcal in order to satisfy the iron needs. This is theoretically possible to accomplish by choosing foods with a high iron content (blood, liver, kidneys, wholemeal bread, green vegetables). Apart from the fact that blood and entrails are in short supply it is unrealistic to assume that the majority of people will change their food habits according to these suggestions. An increase in the dietary iron intake might be most easily accomplished through a fortification programme. Iron fortification of the Norwegian brown-cheese with ferrous sulphate was first performed on an experimental scale in the late 1960's (26). In 1972 the enriched cheese became available to the ordinary consumer.

The most suitable vehicle, however, for iron fortification would probably be flour and bread. In Great Britain the Bread and Flour Regulations (1) require that either reduced iron or ferric ammonium citrate shall be added, if necessary to restore the iron content of flour other than wholemeal, to at least 1.65 mg/100 g flour. In

Denmark 3 mg iron/100 g flour is added in the form of reduced iron to wheat and rye flour (12). In Sweden iron enrichment of flour (up to 7 mg iron/100 g flour as reduced iron, ferrous sulphate or ferrous fumarate) is undertaken on a voluntary basis (14). In the USA the level of iron in "enriched" flour is raised to that of wholemeal flour (3.5 mg Fe/100 g flour). However the US Commissioner of the Food and Drug Administration has proposed an increase of the level of enrichment to ensure a level of 9 mg Fe/100 g flour (3-7). Finch and Mornen (7) emphasize that such manipulation of the public diet carries with it the responsibility for monitoring its effect on the iron balance of the population and on the prevalence of iron deficiency and iron overload.

Based on our previous Hb studies (18) we proposed that the possibility of iron enrichment of flour and bread should be considered also in Norway. It would be necessary however to examine the biological effect of various iron preparations when baked into bread as well as the technological problems relating to the baking process. While iron fortification programmes have been in force for many years in many countries, there appear to have been few thorough attempts to evaluate their effectiveness until recently. Further more the results of these studies have been controversial.

Three long-term feeding trials with ferrous carbonate or ferrum-reductum-enriched bread (5-15%) showed no effect on the Hb values. Studies reported by Stott (23), however are at variance with these negative results. Using prisoners, Stott fed ferrous-sulphate-enriched bread for four months and obtained a considerable increase in the Hb concentration. This work, however has been justifiably criticized because there was no control group and because the absorption of iron from the added ferrous sulphate would have to have been in excess of 30% in order to account for the recorded Hb rise during the first two months of supplementation.

During a long-term trial we fed ferrous-sulphate-enriched bread to 77 women inmates in a mental hospital (24). A control group was given the same bread without fortification. After a 11 week period the mean Hb level increased from 13.96 to 14.32 g/100 ml in the experimental group. This increase of 0.36 g/100 ml was statistically significant at the 5% level. However the

mean Hb increase in the control group was almost as great, but these results were difficult to assess as so many of the controls dropped out. Thus the experiment did not provide convincing proof of the effectiveness of the supplement.

In a recent long-term feeding trial by Elwood et al. (6) the effect of ferric-ammonium-citrate enriched bread was examined. Although the results were not entirely consistent, they indicated a small hematologic effect of the iron supplement.

Balance studies (9) with bread enriched with ferrum reductum, ferrous sulphate or ferric orthophosphate revealed some absorption (on an average 3%) of the added iron. There was no particular difference in the amount of iron absorbed from the three preparations.

In *absorption studies* (24) we have found that ferrous-sulphate-enriched bread gave favourable results, with an increase in serum iron values two hours after the test meal. When reduced iron was used for enrichment, the response was not so pronounced. It was also observed that the added ferrous sulphate was better absorbed from white wheaten bread than from wholemeal bread.

In *radioactive absorption studies* Steinkamp et al. (22) examined the effect of bread enriched with radioactive ferrous sulphate, reduced iron, ferric orthophosphate, and sodium ferric pyrophosphate, and found an equal effectiveness of all four forms of iron used for enrichment. In recent studies Höglund and Reizenstein (10, 11), using more modern technique with a whole body counter found that the absorption of iron from ferrous-sulphate-enriched bread was much higher than when reduced iron was used for enrichment. When fine grain-reduced iron was used to enrich flour the absorption was 50% lower and when a coarser grain-reduced iron was used, it was 85% lower than when ferrous sulphate was used for enrichment.

Animal studies performed by Fritz et al. (3, 20-21) of the biological availability of iron in iron-depleted chicks and rats also indicated that ferrous sulphate has a high relative biological value compared with other iron compounds. It was also found that ferrous sulphate added to a biscuit mix prior to baking was utilized nearly as well as the same quantity of ferrous sulphate added directly to the test diet (8). When these animal repletion studies were compared with human plasma iron responses following test doses of

five sources of iron (ferrous sulphate, reduced iron, sodium iron pyrophosphate, ferric orthophosphate and ferrous carbonate) a similar ranking with ferrous sulphate as the best compound was obtained in both experiments (21).

According to three comprehensive reports on the subject of iron fortification (2, 13, 16) most of the evidence suggests that ferrous sulphate is among the most effective forms.

As a supplement to our previous absorption and utilization studies (24) we felt that a long-term controlled, large-scale community-based feeding trial with ferrous-sulphate-enriched flour and bread should be conducted. Ideally such a trial should evaluate the beneficial effect, if any, on representative samples of the community in terms of a change in morbidity. However, indices of morbidity are relatively insensitive. Changes in Hb levels and other hematological data might be used, as an indirect measure of health, to assess the immediate effect of the fortification programme. The plan was to supply iron-fortified flour and bread to the entire population in one particular community and to compare the changes in certain hematological data of one group of this experimental area with those obtained in a control group from an area without fortification.

MATERIAL AND METHODS

Iron enrichment of flour

Western Norway was chosen for the trial, as in a previous study we had observed somewhat lower Hb values in the western than in other regions of the country (17). Further more, in the western region it was also possible, from practical point of view to establish an experimental area where flour and bread could be fortified, as well as control area without fortification. In cooperation with the Norwegian Grain Corporation, Stavanger was chosen as the experimental area. Here most of the flour used for baking is produced and distributed by one particular mill (Tan Mølle). Bergen was chosen as the control area and here no flour is fortified.

Endorsed by the Nutrition Committee, National Nutrition Council and the Health Directorate, and assisted by E. Melchior, head of the laboratory of the Norwegian Grain Corporation, O. Nilnes, manager of Tan Mølle, and Dr A. Skjerve, head of the Norwegian Cereals Institute at the State Institute of Technology it was decided to add powdered anhydrous ferrous sulphate to all 75% extraction wheat flour which was produced by Tan Mølle to ensure total level of 8 mg Fe/100 g flour.

Previous baking experiments had shown no adverse effects of this iron-fortification procedure on the baking

process as such. There was no unacceptable change in taste, smell or shape of the baked bread. Furthermore, ferrous sulphate is one of the least costly forms of iron available. Thus enrichment is possible without an increased cost of the flour and the baked items. The Norwegian Grain Corporation purchased and dispatched ferrous sulphate in neutral packing to Tan Mølle. The Corporation also defrayed the expenses of the technical equipment necessary for the iron feeding procedure.

In the mill the enrichment process started 1 Dec. following of March 1968. Due to the delay in the bakeries and in the grocery shops, enriched flour and bakery products became available to the public in Stavanger 1-4 weeks later. The enrichment continued until the end of Oct. 1969 i.e. for 20 consecutive months. During this period the mill produced approximately 17 000 metric tons of iron-fortified flour. Samples of the enriched flour at irregular intervals analysed at our Institute in Oslo. The mean iron content was 6.2 mg/100 g flour (31 total variation of 5.0-6.4 mg). The natural iron content of the flour (75% extraction hard flour) as 1.3 mg Fe/100 g. Thus the added ferrous sulphate is equivalent to approximately 5 mg Fe/100 g flour, which is somewhat lower than originally planned. This discrepancy might be due to various technical factors as well as to the evaporation of some water in the so-called subhydrous ferrous sulphate which is used for enrichment.

Our study was performed "blind" without the knowledge of the public. During the period of enrichment there are no complaints concerning the fortified flour or the baked items. It must therefore be assumed that there was no unacceptable change in taste, smell or shape, or deterioration in keeping quality. Thus the iron fortification programme was accomplished without any disclosure or interference from the bakers or the public.

During the experimental period samples are taken of the white wheaten bread which is baked in the bakeries of Stavanger and subsequently sold in the bakers' shops and other stores. Chemical analysis of the enriched bread showed a mean iron content of 3.9 mg iron/100 g bread (total variation 3.1-4.5 mg). The mean iron content of the unfortified variety is 1.2 mg/100 g bread. According to Gjølun (27) the average bread consumption among women today is about 130 g/day. Thus the iron intake per day from the enriched bread would be 5.1 mg, in contrast to only 1.6 mg from the unfortified variety i.e. an addition of 3.5 mg.

Material and experimental procedure

The experimental as well as the control group should be recruited from apparently healthy women fit for work, aged approximately 25-40 years, i.e. the age group with the highest prevalence of iron deficiency anemia. As blood samples had to be taken before the start of the experiment as well as during the enrichment period, it was logical to cooperate with industrial medical officers who had direct access to a relatively large number of young women fit for work. In Stavanger Dr E. Tryveth, head of the Municipal Industrial Medical Office, and in Bergen Dr E. Mørk, head of the Industrial Medical Office of Televerket, Trygdehuset and Kjennerbust, participated

Table I Time of examinations and the number of women who participated

In the experimental group 1 woman and in the control group 9 women were included after examination I. The figures within parentheses indicate how many of these participated in examinations II, III and IV.

	Examination				Examined all four times
	I	II	III	IV	
Time	{ Feb./ March 1968	{ Oct./ Nov. 1968	{ Feb./ March 1969	{ Oct. 1969	
Experimental group	202	134	160 (1)	150	136
Control group	215	195 (7)	172 (9)	146 (6)	137

In the study. They were informed about the enrichment experiment as well as the objects of the study. The participating women, however, were told that the blood examinations were part of the assessment of blood values in Norwegian women.

Assisted by the staff of the two industrial medical offices it was possible to establish an experimental group in Sta.anger and a control group in Bergen.

Blood samples were taken before the start of the enrichment experiment (Feb./March 1968) and on three occasions during the study (Oct./Nov. 1968, Feb./March 1969 and Oct. 1969).

At the time of the first examination (examination I) questionnaires were completed in order to assess the intake of supplementary iron during the last 12 months as well as conditions associated with blood loss including blood donation. The time of the last menstrual bleeding and the average duration of the bleedings were registered. Any previous or present serious as well as chronic disease was also recorded and, if necessary, additional information from the industrial medical officer as included.

Similar questionnaires concerning the interval since the last examination were also completed in connection with examinations II, III and IV. The use of p-pills was recorded at examinations III and IV.

Hematological method

The determinations of Hb concentration and Hct were performed in blood samples taken from the fingertips by

Table II Mean Hb, Hct and MCHC values in the two groups at the start of the study (examination I)

	Experimental group (202)	Control group (215)
Hb (g/100 ml)	11.20	13.12
Hct (%)	39.24	39.03
MCHC (%)	33.64	33.62

pricking with lancets. From each individual 1 sample was taken, one from the right and one from the left hand, and the mean values of Hb and Hct in each individual were used in the further analysis and in the calculation of MCHC (mean corpuscular hemoglobin concentration).

The Hb determinations were performed by the cyanmethemoglobin method with photoelectric reading in Lincom Junior photoelectric colorimeter. The colorimeter was calibrated against standardized cyanmethemoglobin solutions. The Hct was measured in heparinized capillary tubes after centrifugation in a Hct centrifuge (AB L. Ljungberg & Co., Stockholm). The analytical methods were in principle consistent with the standard procedure used at our Institute during the past several years (18).

To secure conformity the same colorimeter and the same Hct centrifuge were used throughout the study. The same well trained nurse took all the blood samples and performed all the readings in both the experimental and the control group. Thus there should be no obvious methodological bias in the comparison of the experimental and the control groups.

RESULTS

The time of each of the four examinations and the number of women participating are presented in Table I. At the start of the experiment the experimental group consisted of 205 women and the control group of 216 women. However three of the women in the experimental group with Hb values below 11 g/100 ml were on ethical grounds excluded from the study. The industrial medical officer was informed and was recommended to start iron therapy in these cases. One woman in the control group was also excluded as her fingers were deformed to such an extent that blood sampling was impeded.

Thus 202 women in the experimental group and 215 in the control group were included in the experiment. The age of the experimental group was on an average 32.0 years (total variation 24-40), and practically the same in the control group 31.8 years (24-43).

Many of the women originally included in the trial (experimental and control groups) were not present at all three consecutive examinations. These women will subsequently be called *drop-outs*. On the other hand, one woman was erroneously included in the experimental group and nine women in the control group after examination I. Thus only 136 women in the experimental group and 137 in the control group were examined all four times.

At the start of the experiment there were only

Table III. Hb, Hct and MCHC values (mean \pm S.E.) in the women who participated in all four examinations

Examination	Hb (g/100 ml)		Hct (%)		MCHC ()	
	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group
I	13.1 \pm 0.06	13.12 \pm 0.06	39.18 \pm 0.19	39.12 \pm 0.17	33.76 \pm 0.12	33.64 \pm 0.10
II	13.39 \pm 0.07	13.44 \pm 0.07	39.22 \pm 0.18	39.71 \pm 0.18	33.99 \pm 0.11	33.79 \pm 0.10
III	13.17 \pm 0.06	13.14 \pm 0.06	38.87 \pm 0.20	39.12 \pm 0.17	33.82 \pm 0.10	33.47 \pm 0.11
IV	13.11 \pm 0.08	13.05 \pm 0.08	39.12 \pm 0.22	39.27 \pm 0.21	33.57 \pm 0.13	33.21 \pm 0.11
Difference IV-I	-0.10	-0.07	-0.06	-0.15	0.19	-0.43

minor differences between the experimental and the control groups with regard to the mean Hb, Hct and MCHC values (Table III). The relative distribution of the Hb concentration in the two groups was also nearly identical (Fig. 1). It should be noted that the mean Hb values in both groups were lower than previously observed in Norwegian women (19).

The changes in the mean Hb, Hct and MCHC values in both groups during the study are presented in Table III. Only women who participated in all four examinations are considered here. There was no particular tendency to any appreciable change in any of the parameters; most mean values at the end of the 20 months trial were practically on the same level or slightly lower than observed at the start of the experiment. Thus there was no noticeable difference between the experimental and the control groups.

In the experimental as well as in the control group a large number of the women who participated at all four examinations gave information of ailments, non-normal physiological conditions or therapeutic regimes which would, or at least might, influence the hematological values. These conditions are listed as "exclusion diagnoses" and presented in Table IV. In the Table the "exclusion diagnoses" are also given for another 39 women in the experimental group and 41 in the control group who dropped out during the study. As shown, iron therapy was very common in both the experimental and the control group before as well as during the study. On the other hand, iron loss in connection with blood donation, pregnancy delivery and abortion was also frequently encountered. Other "exclusion diagnoses" were the use of p-pills and a number of other conditions (surgical treatment,

pathological bleedings, polyarthrits and other collagen diseases, the use of antirheumatic drugs, serious infections, etc.).

Thus only 46 women in the experimental group and 72 in the control group completed the experiment without any exclusion diagnosis. The mean values of Hb, Hct and MCHC in these women are presented in Table V. Although there are irregular fluctuations in the hematological parameters in both the experimental and the control group during the experimental period, the seem to be only small differences between the two groups. In the experimental group there was a small increase in both Hb and Hct from examination I to IV whereas in the control group Hb

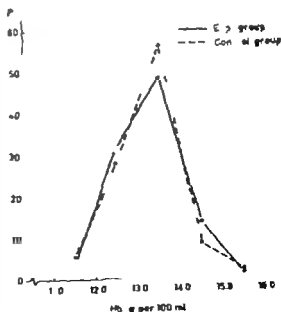


Fig. 1 Relative distribution of the Hb concentration at the start of the experiment.

Table IV Number of various "exclusion diagnoses" in the two groups

Exclusion diagnosis	Present at all four examinations		Drop-outs		Total	
	Experimental group (n=90)	Control group (n=65)	Experimental group (n=39)	Control group (n=41)	Experimental group (n=129)	Control group (n=106)
Iron therapy						
Before the study	69	32	23	14	92	46
During the study	38 } 107	37 } 69	18 } 41	18 } 32	56 } 148	55 } 101
Blood donor						
Before the study	9	5	5	6	14	11
During the study	12 } 21	3 } 8	2 } 7	2 } 8	14 } 22	5 } 16
Diets dry during the study	9	10	5	3	14	13
Abortion during the study	3	1	1	0	4	1
Pregnant during the study	10	8	9	19	19	27
P-pili	11	5	3	0	14	5
Others	14	14	6	4	20	18
Total	175	115	72	66	247	181

was unchanged and Hct showed only a very slight increase. In the experimental group the MCHC values were practically unchanged but decreased slightly in the control group.

An extra supply of iron, however would not necessarily imply a noticeable increase in the mean Hb and other hematological values of the group as a whole, as most of the examined individuals might already have optimal or near-optimal values in order to make the test more sensitive it was necessary to examine those women with hematological values at the lower end of the distribution curve.

Four women in the experimental group and six in the control group with initial Hb values < 12 g/100 ml were selected for this evaluation. Two of the four women in the experimental

group, however had some blood loss during the experimental period (bleeding hemorrhoids, operative treatment with some bleeding) whereas all six in the control group had no interfering conditions which might influence the hematological values. By chemical analysis of the bread supply it was confirmed that the four women in the experimental group really consumed iron-fortified bread. The mean Hb, Hct and MCHC values of these women are given in Table VI. In spite of the presence of bleeding conditions in two of the four women in the experimental group, there was a noticeable increase in the Hb values during the experimental period from 11.81 g/100 ml at the start of the experiment to 12.71 g/100 ml at the end of the period i.e. an increase of 0.90 g/100 ml. In the control group there was no sig-

Table V Hb, Hct and MCHC values (mean \pm S.E.) in women without "exclusion diagnosis"

Examination	Hb (g/100 ml)		Hct (%)		MCHC (%)	
	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group
I	13.47 ± 0.11	13.22 ± 0.08	39.85 ± 0.29	39.49 ± 0.23	33.87 ± 0.24	33.64 ± 0.13
II	13.65 ± 0.11	13.58 ± 0.09	39.87 ± 0.29	40.12 ± 0.23	34.11 ± 0.19	33.79 ± 0.11
III	13.44 ± 0.09	13.27 ± 0.08	39.76 ± 0.31	39.43 ± 0.22	33.78 ± 0.16	33.56 ± 0.12
IV	13.58 ± 0.12	13.22 ± 0.09	40.48 ± 0.34	39.76 ± 0.24	33.78 ± 0.25	33.18 ± 0.11
Difference IV-I	+0.11	0	+0.63	+0.27	-0.09	-0.46

Table VI. Mean Hb, Hct and MCHC values in women with an initial Hb value <12 g/100 ml

Examination	Hb (g/100 ml)		Hct (%)		MCHC (%)	
	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group
I	11.81	11.90	36.6	36.2	32.25	32.93
II	12.29	12.51	36.3	37.8	33.93	33.15
III	12.44	12.25	37.3	36.7	33.45	33.42
IV	12.71	11.91	37.5	37.0	33.91	32.19
Difference (IV-I)	+0.90	+0.01	+0.9	+0.8	+1.66	-0.74

tained increase in the Hb values. After a transitory increase (examination II) the Hb values decreased and reached the initial value at the end of the experimental period. The increase in the Hb value of the experimental group was significantly different ($p=0.05$) from that observed in the control group. There was also a considerable increase in the mean MCHC values of the experimental group during the trial, in contrast to what was observed in the control group, the final MCHC value of which was lower than the initial value. Regarding Hct there was an increase of 0.9% in the experimental group and 0.8% in the control group. The small difference between the groups was not statistically significant.

DISCUSSION AND CONCLUSIONS

It is necessary to consider two fundamental aspects of our experiment with ferrous-sulphate-enriched flour before wider conclusions are inferred.

Firstly was it a reasonably sensitive trial? The mean Hb concentration in the experimental group at the start of the trial was 13.20 g/100 ml and this value was about 1 g below the previously proposed mean normal value (14.3 g/100 ml) in Norwegian women of comparable age (19). However this normal value was based on blood samples obtained by venepuncture in contrast to the present bread study in which blood samples were taken from the fingertip by pricking with lancets. A small methodological study revealed that, when the results of the industrial nurse from the previous study (19) using venepuncture were compared with the results of the nurse using pricking of the fingertip in the same female subjects, the mean venepuncture value was 0.45 g/100 ml above the fingertip value. Thus the max-

imal expected mean increase in Hb concentration in the experimental group of the present study would be only approximately 0.5 g and not 1 g/100 ml as originally assumed.

When the women with exclusion diagnoses were excluded from the material the mean Hb value at the start of the experiment was 13.47 g/100 ml, with values of 13.65, 13.44 and 13.58 at the following three examinations. The differences between these values and the expected mean normal value of 13.85 g/100 ml, when corrected for the difference between venepuncture and finger pricking (14.30-0.45 g/100 ml), were rather small. Furthermore, the mean Hb value in the experimental group was at the start of the experiment somewhat higher (13.47 g/100 ml) than that observed in the control group (13.22 g/100 ml). This difference might be due to the apparently more liberal practice of iron medication in Stavanger (Table IV), probably leading to a lower prevalence of untreated iron deficiency in the experimental group already at the start of the experiment. Thus the mean Hb value in the experimental group, before as well as during the iron fortification period, was rather close to the mean normal value, and therefore it might be assumed that the sensitivity of the trial was not sufficient to test the effect of the iron-fortification programme.

However the results obtained in the women with the lowest circulating Hb values (Hb <12 g/100 ml) suggested that there really was an increase in Hb concentration during the fortification period. Although the number of women in both the experimental and the control groups was small, the encountered difference between the two groups was statistically significant.

Secondly our trial submitted a prophylactic

measure to a therapeutic test, i.e. a rise in the Hb level was expected. Elwood et al. (6) have recently used a more realistic prophylactic design. All the anemic women in a large community sample were identified and given sufficient conventional treatment with oral iron to raise their Hb level. A random sample of the treated women were given bread containing ferric ammonium citrate (2.7 mg Fe added per 100 g flour). The effect of the iron in the bread was assessed by the degree to which it prevented a subsequent decrease in Hb concentration. Although the results were not entirely consistent, they suggested that the iron supplement had some effect, albeit small, on the Hb concentration whether given in bread or in tablets.

Although our experiment with ferrous-sulphate-enriched flour failed to give conclusive evidence of the hematologic effect of the iron supplement, the results obtained in women with Hb <12 g/100 ml suggest that the added iron is absorbed and utilized for Hb synthesis.

As iron deficiency is rather prevalent in the Norwegian population due to an insufficient supply of dietary iron, we have recommended to the Norwegian health authorities, based on the results of the present study and the findings by other workers, that all white flour should be enriched with ferrous sulphate. Furthermore it seems reasonable to follow the proposal of the US Commissioner of the Food and Drug Administration and thus achieve a total level of 9 mg Fe/100 g flour.

ACKNOWLEDGEMENTS

Financially supported by the Norwegian Grain Corporation and by research grants from Nansenfondet and J. L. Thedensens Tobakstiftelse. Joh. H. Andreasen assisted in the lab.

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PLATELET COUNTS IN MOTHERS AND THEIR NEWBORN INFANTS WITH RESPECT TO ANTE PARTUM ADMINISTRATION OF ORAL DIURETICS

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Abstract. In order to investigate a possible effect of diuretics on maternal and neonatal thrombocytopoiesis, platelet count was performed on 200 mothers and their offspring. One hundred of the mothers had received treatment with oral diuretics for at least 3 weeks prior to the admission, while the remaining 100 had not received any diuretic agent. Transitory thrombocytopenia (defined as a platelet count below $100\,000/\text{mm}^3$) was observed in 6% of treated mothers, 9% of control mothers, 1% of infants born to treated mothers and 7% of control infants. No case of thrombocytopenia was associated with clinical signs of a bleeding tendency. It is concluded that short time ante-partum therapy with oral diuretics in this series did not affect the platelet counts in mothers or their newborn infants.

Oral diuretics are widely used in the treatment of oedema and hypertension during the last month of pregnancy. Scattered reports have appeared on thrombocytopenia due to administration of these drugs in adults (1, 2, 3, 9, 10, 12). In 1964 Rodriguez et al. (14) suggested a relationship between severe thrombocytopenia observed in seven newborn infants and maternal ante-partum diuretic therapy. However, more recently Robbs (13) and Merenstein et al. (11) could not demonstrate significantly lower platelet counts in 29 and 37 newborn infants, respectively, whose mothers had been treated with these drugs as compared with control infants. The aim of the present work was to re-investigate in a larger group of patients the question whether maternal ante-partum diuretic therapy is associated with neonatal thrombocytopenia.

MATERIAL AND METHODS

Platelet counts were performed in two groups of mothers and their infants. The first group consisted of women (and infants) who had received oral diuretics prior to

the delivery and the second group of women (and infants) who had not received these drugs.

The diuretics used were bendroflumethiazide (60%), furosemide (15%), polythiazide (14%), chlorothalidone (5%), chlorazamide (4%) and chlorothiazide (2%).

On the day of admission to the delivery ward platelet counts are performed on finger-prick capillary blood in 200 cases 4-20 hours prior to delivery. One hundred of them are consecutive subjects who had received oral diuretics continuously for at least 3 weeks prior to the admission. All patients were considered clinically well apart from the tendency to normotensive fluid BP in all mothers was less than 150/90 mm Hg and multivitamin preparations were the only extra medications, and did not exclude the patient from the study. Deliveries were by the vaginal route. All newborns included had a birth weight above 2500 g and showed an uneventful postnatal course. The patient admitted to the delivery and immediately subsequent to treated case and he had not received any diuretics during the current pregnancy was used as control case. In all 200 infants platelet counts, by the technique of Kristensen (8), were performed on the morning after birth (at 2-26 hours of age). The platelet counts are reported as the mean values \pm standard error of the mean (S.E.M.).

RESULTS

The mean platelet count for the control group of women was $168\,000 \pm 5\,000/\text{mm}^3$ and for the women who had received oral diuretics $159\,000 \pm 4\,000/\text{mm}^3$. The mean platelet count for the control infants was $242\,000 \pm 8\,000/\text{mm}^3$ and for the infants whose mothers had been treated with oral diuretics $240\,000 \pm 8\,000/\text{mm}^3$. There was no statistically significant difference in platelet counts between either the two groups of mothers or the two groups of infants studied.

Table I gives the mean maternal and infant platelet counts with respect to the duration of ante-partum diuretic therapy. In 65% of the cases

Table 1. Maternal and infant platelet counts (mean \pm S.E.M.) with respect to the duration of anti-partum diuretic therapy

Duration of diuretic therapy (weeks)	No. of cases	Platelet count/mm ³	
		Maternal	Infant
3-6	63	156 000 \pm 5 000	237 000 \pm 10 000
7-10	21	163 000 \pm 13 000	239 000 \pm 12 000
11-14	11	158 000 \pm 7 000	247 000 \pm 35 000
15-18	3	173 000 \pm 9 000	268 000 \pm 70 000
Total	100	159 000 \pm 4 000	240 000 \pm 8 000

the therapy did not exceed 6 weeks. As is evident from Table 1 there was no tendency to lower platelet counts in cases where the therapy was of longer duration.

A platelet count below 100 000/mm³ was observed in nine of the control women and in six of the women who had received oral diuretics. All these 15 women had a count above 100 000 on the morning after delivery. In no case was a platelet count below 80 000/mm³.

Seven of the control infants, but only one of the infants whose mothers had received oral diuretics had a platelet count below 100 000/mm³. All of these eight infants had counts above 100 000 on the second day after birth. No statistical correlation was found between platelet counts in mother and child. No increased bleeding tendency was noted in any case.

COMMENT

It has been suggested that, both in the adult (9) and in the newborn infant (14), the thrombocytopenia sometimes encountered in association with administration of oral diuretics is due to bone marrow toxicity of these drugs with a depression of platelet production. As these drugs are readily transferred across the placental membranes (4), the same mechanism underlying an adverse drug reaction might be expected in both the mother and the foetus. Recently however the possibility of an immunological aetiology of the thrombocytopenia has been proposed (3, 6, 7).

Thrombocytopenia as defined by a platelet count below 100 000/mm³ was observed in 9% of control mothers and in only 6% of mothers treated with diuretics. Among infants, 7% of un-

treated cases were thrombocytopenic according to the definition given while this occurred only in 1% of infants born to treated mothers. These findings strongly suggest that there is no correlation between the occasional finding of low platelet count and diuretic treatment. This is in accordance with the results presented by Robbe (13) and Merenstein et al. (11), and it may thus be concluded that the toxicity of these drugs seems to be low as regards foetal thrombocytopenia. Rodriguez et al. (14) found normal platelet counts in every mother of the thrombocytopenic infants. It therefore seems reasonable to assume an unusual sensitivity of the foetal bone marrow to these agents if a thrombocytopenia is the result of anti-partum maternal use of oral diuretics.

In the present work the anti-partum drug therapy was of relatively short duration, which is the rule in obstetric practice. In 65% of the cases the duration of therapy did not exceed 6 weeks. It might well be that more prolonged therapy would increase the risk for development of harmful drug reactions, but in our series no positive correlation was found between duration of therapy and maternal or infant platelet count (Table 1).

It is concluded that short-time anti-partum administration of oral diuretics in this series did not affect the platelet count either in the pregnant woman or in the foetus. However as in all pharmacotherapy due to the potential risk of undesirable drug reactions, the institution of oral diuretics to pregnant women must be done on correct indications and not be considered as a routine action.

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Congress Announcements

International Symposium on Wound Healing will be held at the new premises of the Medical Faculty of the Erasmus University Rotterdam, the Netherlands, April 8-12, 1974.

Deadline for abstracts. Dec. 15 1973. Papers of max. 300 words to be submitted to the Secretariat.

Information. The Secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague the Netherlands.

International Symposium on Pulmonary Circulation II will be held in Prague, Czechoslovakia, June 17-19 1974.

Topics: 1. Long-term development of pulmonary hypertension in chronic obstructive bronchopulmonary disease, 2. Pulmonary hypertension at altitude, 3. Pulmonary circulation in left heart failure.

Preliminary application form can be obtained by The Czechoslovak Medical Society J. E. Purkyně Sokolská 31 120 26 Prague 2, Czechoslovakia.

The international prize for modern nutrition of Sfr. 15 000 will be awarded in Sept. 1974 by the Central Union of Swiss Milk Producers, Berne, to a scientist from one of the following countries, members of the International Dairy Federation. Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, Czechoslovakia, Denmark, Finland, France, India, Ireland, Israel, Italy, Japan, Kenya, Luxembourg, Netherlands, New Zealand, Norway, Poland, South Africa, Spain, Sweden, Switzerland, United Kingdom, USSR, West-Germany.

The subject chosen for the 1974 prize is *Milk and lactation*.

International Symposium on Recent Advances in the Assessment of the Health Effects of Environmental Pollution will be held in Paris, France, June 24-28 1974.

Organizers. the Commission of the European Communities, the United States Environmental Protection Agency and the WHO.

Main subjects. 1. Exposure assessment of general and selected population groups, 2. Metabolic aspects of pollutants in exposed individuals, 3. Evaluation of suspected or observed health effects from exposure to environmental pollutants.

Submission of papers. Before Nov. 15 1973.

Information. The Secretariat of the Symposium "Environmental Health" Health Protection Directorate Commission of the European Communities, 29 rue Aldringen, Luxembourg (Grand Duchy).

All scientists (chemists, physicians, biologists) who have worked in this field are eligible. Applications should be sent to the President of the jury Professor M. Demole Unité de Diététique, Hôpital Cantonal, CH 1 11 Genève 4 until Jan. 31 1974 with 3 copies of a) curriculum vitae b) list of works, c) reprints of 2 or 3 papers on the theme of the prize, published in the last 5 years (no typewritten papers). These documents should be written in English, French or German or should be accompanied by a translation into one of these 3 languages. (They will not be returned to the authors.)

EDITORIAL

LOST IN THE HODGKIN MAZE?

Our knowledge of Hodgkin's disease has increased rapidly during recent years. Only a decade ago many—or most—clinicians regarded Hodgkin's disease as a rather uniformly malignant process and patients were treated accordingly—in hospitals without any specialized knowledge.

The almost explosive development that we have now witnessed in the Hodgkin field has complicated things to such a degree that the expression "the Hodgkin maze" has been coined (7). The new information concerns such different aspects as 1) new ideas regarding aetiology and epidemiology, 2) the demonstration of disturbances in the immune system, 3) a new staging system with definite implications for treatment and prognosis, 4) the concept of laparotomy and splenectomy for better staging, and finally 5) new effective techniques for high energy radiation and the adoption of the aggressive multitasking combination chemotherapy. It goes without saying that it is impossible for any single individual to keep abreast of this development and warnings have already appeared that the new knowledge is being mishandled.

Vianna et al. (21) and Order and Hellman (15) have suggested that Hodgkin's disease is caused by a virus—with low virulence and long incubation time. Vianna arrived at his hypothesis at least partly from his description of clustering of cases of Hodgkin's disease among Albany High School students in the USA (20). A similar—although less marked—finding had been reported from the Manchester area in Great Britain (2) and quite recently also from Ohio, USA (9).

Different subdivisions of Hodgkin's disease with regard to histology have long been made, but have been of interest almost exclusively for pathologists—the different types have remained enigmatic and without clinical implications. The situa-

tion was radically altered when Lukes and Butler (11) published their grading in 1966, originally into six later modified to four types that showed distinct differences with regard to malignancy and survival. The best prognosis follows the type with lymphocytic-histiocytic proliferation with few Sternberg Reed cells, and the worse prognosis the form with lymphocytic depletion. Nodular sclerosis in many respects takes an intermediate position.

This has led also to a concept in which the basic neoplastic process is the Sternberg Reed cell and the associated histological components are regarded as host factors, showing a more (lymphocytic proliferation) or less (lymphocytic depletion) pronounced host defence against the aggressor (10). Other indications of involvement of the immune system are the finding of a tumour-associated antigen (14) as well as the demonstration of a defect in cell-mediated immunity displayed by the vast majority of Hodgkin patients (1). Significantly increased risks of development of other malignant tumours have been found in a large series of patients treated for Hodgkin's disease (3). Furthermore, persisting Hodgkin tissue has been demonstrated in patients who many years previously had been treated for and clinically cured of Hodgkin's disease, now dying from other causes. A balance may thus be established between the neoplastic process and the defence mechanisms of the host (18) in such a way that the neoplasia is kept under control. Studies of long-term survivals—10 years or more—have been reported (5), again emphasizing host factors related to cellular immune mechanisms as being of major importance for determining in which patients remissions will be prolonged.

To complicate matters, the 4 *markers* (11) have been identified in other conditions (12, 13). A 11

as malignant, such as infectious mononucleosis (12), Burkitt lymphoma (22) and other malignant lymphomas (17).

The histological grading has definite relations to staging, which previously has played a less important role in Hodgkin's disease than in many other types of malignant disorders. The system proposed in 1965 (Rye Conference) has been widely used during recent years, but has now been found to be inadequate. A recent meeting (April 1971) at Ann Arbor (19) USA therefore, has further developed the concepts and suggested a much more elaborate system of staging—with regard to clinical as well as to histopathological findings. The new system has been constructed mainly because of the demonstration, by laparotomy and otherwise, of much more diffuse spread of the disease process than was previously anticipated. The system looks rather complicated—a patient might be classified as

CS IVB₁PS IV₂ x

indicating clinical stage IV with widespread disease, with general symptoms, gross involvement of lung and liver and pathological stage IV due to positive liver biopsy although the bone marrow biopsy was negative.

A special reason for discussing the recent developments in Hodgkin's disease is the question of laparotomy that was introduced mainly for the purpose of staging. It turned out that the gross appearance of the spleen was not sufficient to tell whether splenic involvement existed or not, and thus splenectomy was added. The latter step also made unnecessary any irradiation of the spleen as a part of subsequent therapy and eliminated the potential risks of irradiation damage to basal parts of the left lung and left kidney.

After the first publications laparotomy and splenectomy were widely adopted and many patients were operated upon—but negative reports soon appeared, mainly concerning overwhelming infections after such operations (8).

The immunological defects discussed above may imply that patients with Hodgkin's disease, like those with chronic lymphocytic leukaemia, are more susceptible to infections. In fact, all splenectomized patients have been thought to suffer from a diminished resistance to infections. A study of the recent literature, however, does not support this widespread idea (4).

It is important to realize that laparotomy—with or without splenectomy—must be undertaken only as a part of a careful plan to help the individual patient. No patient should be operated upon if it is not clear that he or she will have a fair chance of benefiting from the operation and from better planned therapy. The role of splenectomy in this connection has to be especially evaluated—there may be advantages in splenectomy per se, but the important indications are diagnostic (staging) and technical (less radiation). It is impossible today to speculate about the future role of laparotomy and splenectomy as part of the Hodgkin treatment. Much more knowledge needs to be accumulated before answers can be given to the question that have arisen from recent developments.

Finally treatment has become more effective. Radiation is the treatment of choice in the early stages and may now be applied very effectively to known sites of disease as well as prophylactically to adjacent or to "all" lymph node sites (for references, see 13). Chemotherapy has resulted in complete remissions in as many as 80–85% of patients in later stages through the use of treatment programmes generally combining four "anti-cancer" drugs in varying types of cycles (5, 16). Lately too combinations of radiotherapy and chemotherapy have been tried in several centres (13).

All of these aspects—only briefly touched upon—clearly show how complicated the situation has become. It should be evident that patients with Hodgkin's disease must be treated in specialized centres—though at present this is not everywhere the case. This is especially important in order to prevent the new ideas and concepts from being adopted too hastily. Today this too eager acceptance of the new ideas seems to be a real danger to the development of our knowledge—and to the individual patient.

We certainly have a long way to go before we can get out of the Hodgkin maze—perhaps we are beginning to see the distant lights from the exit.

L. E. Böttiger

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BOOK REVIEW

Hepatology. By Tygstrup & Whistler III cr. 155—Munksgaard, Copenhagen, Denmark, 1973.

This monograph by Scandinavian, chiefly Danish, authors contains a wealth of interesting information, as may be expected when we remember the leading part played by the Copenhagen liver school ever since the pioneer work of Roholm and Iversen. As a matter of fact this number of *Acta* contains a paper that illustrates the continuing interest in and work on the advancing front of hepatology in Copenhagen (p. 485).

The publisher Munksgaard, has started a series called postgraduate books for doctors, and this is really a good description of this first volume. It is equally well suited for the young doctor who wishes to continue and broaden his education and for the advanced man who needs to keep his knowledge up to date. The volume is very decidedly clinical and will be of great help to Scandinavian gastroenterologists.

The fundamental biochemical processes have been treated in masterly fashion by a biochemist, who has also been able to relate metabolic happenings to the ultrastructure of the liver cell. This is a sound introduction to the subsequent chapters. Excellent contributions from such authorities as Tygstrup, Whistler and Bjørnsohn follow. The latter presents clear statements of the complicated facts regarding the clinical importance of the Australia antigen. Tygstrup and his group: Rigsbohlpladet attack the problems of chronic hepatitis and cirrhosis from the bedside in a department of medicine. Several surgeons cover such subjects as diseases of the bile ducts, including an excellent chapter by Andreassen, or, mirabile dicta, in some purely metabolically oriented treatises on the composition and chemistry of bile acids and the formation of bile stones by Scherwitz of Odenseburg. In the last chapter one misses the epoch-making investigations from the Mayo Clinic and their implications for the non-surgical treatment of cholelithiasis. The results were obviously too recent to be included. More surprising is

the omission of fetin (α -fetoprotein) in the chapter on liver tumours. This has become an important diagnostic tool for everyday use.

Bolten gives the latest information regarding the value of arteriography with pictures that are on the whole very good in spite of their limited format. The importance of the scintigram is also discussed, but one awaits an evaluation of the gammacamera that will probably be the method of the future.

There are also many well written chapters on metabolic problems. One of the most important seems to me the discussion by Orrenius on detoxification of drugs and other chemicals. Many extremely rare inborn errors of metabolism are treated, even if their connection with the liver is rather tenuous. It is therefore surprising that not a word is said about the porphyrias. The group of hepatic porphyrias really belongs to hepatology and both porphyrinuria and its connections with alcoholism and intermittent δ , in which early diagnosis may be life-saving, should have been treated in some detail.

As this first edition of the book, it is hoped, will be widespread in doctor's studies, I expect that a new edition will soon appear with some additions. The book is highly recommended, it deserves to be the first volume in this new series of postgraduate monographs planned by the publisher.

Two things must be regretted, however. The first is the price, which makes it difficult for private doctors to buy the volume. The price is, of course, partly explained by the small number of copies that can be sold in Scandinavia, but it also illustrates the influence of taxes. The price in Denmark is 155 Danish crowns. In Sweden it is 162 Sw cr. The second is the language. It seems pity that such an excellent work should not have been translated into an international language. In view of the many excellent Anglo-Saxon textbooks on hepatology already published it seems as if a German translation might be most needed.

Jan O. Wahlström

FATTY LIVER PERSISTING FOR UP TO 33 YEARS

A Follow-up of the Iversen/Roholm Liver Biopsy Material

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Abstract. Re-evaluation of 890 consecutive liver biopsy specimens from the period 1938-56 has been done and fatty liver diagnosed in 59 patients. A follow-up examination as performed on 58 of these patients, their time of observation in respect to long-term prognosis of fatty liver varying from 16-33 years, half of the patients being alcoholics. Evaluation of the liver was done on the basis of microscopy from repeated biopsy or autopsy (18 cases), on postmortem gross examination (19 cases), on clinical and laboratory findings (4 cases) or on death certificate (17 cases). In the follow-up period cirrhosis of the liver was diagnosed in two patients only one being an alcoholic. A certain degree of fatty liver was shown in nearly all cases in which histology had been performed. Our results support the hypothesis that steatosis and cirrhosis represent the various forms of response from the liver rather than coherent stages in the development of alcoholic liver damage.

In 1939 Iversen and Roholm (1) presented the first results of a liver biopsy technique and demonstrated the value of this method in the diagnosis of liver diseases.

We have re-evaluated the biopsy specimens collected by Iversen 1938-56 and traced the patients with fatty changes with the purpose of elucidating the long-term course of fatty liver. Due to the very long period of observation, the resulting follow-up material may perhaps throw some light upon the possible transition of fatty liver into hepatic cirrhosis.

MATERIAL AND METHODS

The primary material consists of 890 consecutive percutaneous liver biopsies performed in Medical Department III, Kommunehospitalet, Copenhagen, in the period 1938-56, during which P. Iversen, as the head of the department, All the biopsy specimens have been re-evaluated by one person who had no knowledge of the patients

clinical diagnoses. For technical reasons no diagnosis could be established in 97 cases (11%). In 59 cases (7% of the remaining material) fatty liver was diagnosed. These patients represent the material of the present study (Fig. 1).

Patients with cirrhosis (defined as nodular regeneration and fibrosis), suspected of cirrhosis, with Mallory bodies, signs of cholestasis of the chlorpromazine type or extrahepatic cholestasis, signs of viral hepatitis, malformations, neoplasms or vascular disorders, are excluded. The fatty changes were classified in the following manner: + the biopsy specimen contained fatty vacuoles, but on an average not less than one third of the cells; ++ the specimens contained fatty vacuoles in one third or more but in less than two thirds of the cells; +++ the specimens contained fatty vacuoles in two thirds or more of the cells.

A follow-up of the 59 patients was performed at the beginning of 1972 (16 to 33 years after the primary biopsy) by search through the various Public Records Offices in Denmark, The Office of Medical Statistics, The National Health Service, The National Archives, The Municipal Archives, Copenhagen University and general hospitals, by inquiries to general practitioners, and by individual personal contact. All the 59 patients were traced, except one person who had emigrated. Fourteen patients were alive at the time of the follow-up examination. In nine of these patients repeated liver biopsy a.s.m. (Mallory's) was performed. Four patients refused biopsy and one, as mentioned, had emigrated. The remaining material was evaluated on the basis of available information such as autopsy (18) or without microscopy of the liver), clinical and laboratory examinations or death certificates as shown in Fig. 1 and Table I. The presence of alcoholism was evaluated from the case histories. Alcoholism was defined as an ethanol intake above 50 g/day during the preceding 5 years.

RESULTS

Table I shows some information about 18 patients in whom microscopy of the liver had been performed primarily (biopsy) as well as on follow-up

Table 1. *Histological follow-up after 1-33 years of observation in 18 patients with fatty liver*

Age (y.)	Sex	Chronic alcoholism	Observation period (y.)	Grade of fatty liver		Main cause of death based on autopsy
				Primary biopsy	Latest microscopy	
33	♂	NP	31	++	++	Alive
36	♀	NP	28	+	0	Alive
26	♂	NP	26	+	++	Alive
51	♂	NP	24	++	0	Adenocarcinoma of the prostate
41	♂	F	24	++	++	Cancer of the lungs
37	♂	F	21	+	+++	Pulmonary embolism
58	♀	NP	22	++	+	Alive
51	♂	F	19	++	+	Alive
57	♂	F	16	++	+	Alive
60	♂	NP	16	++	Cirrhosis	Hepatic coma
35	♀	F	15	++	+	Alive
56	♂	F	14	+++	+	Alive
59	♀	NP	13	++	+	Alive
52	♂	F	10	++	Cirrhosis	Hepatic coma
64	♂	NP	9	+	+	Heart failure
39	♀	NP	5	+	++	Pneumonia
60	♂	NP	1	+	0	Barbiturate intoxication
51	♂	F	1	++	++	Cancer of the tongue

NP = not present, F = present.

examinations (biopsy or autopsy). The patients are listed in order of the duration of observation, which varies from 1 to 33 years (median 16).

Eight of the patients in this group were alcoholics. The duration of observation in this subgroup was 1-24 years. In one patient repeated

biopsy showed cirrhosis of the liver. The seven other alcoholics still had a varying degree of steatosis of the liver.

Of the ten patients with non-alcoholic steatosis one developed cirrhosis of the liver and in three the liver appeared normal on repeated biopsy. In

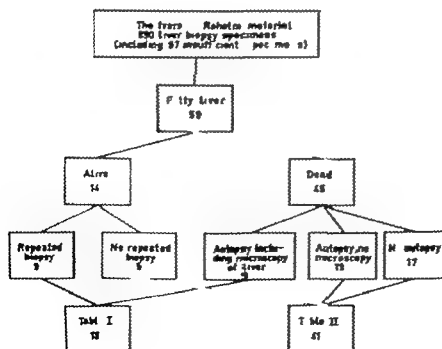


Fig. 1. Fifty-nine patients with fatty liver and the type of follow-up.

Table II. *Non-histological follow-up in 41 patients with fatty liver*

Age (y)	Sex	Chronic alcoholism*	Observation period (y)	Grade of fatty liver & primary biopsy	Latest liver diagnosis	Diagnosis based on			
						Death certificate	Clinical observations	Autopsy	Main cause of death
37	♂	NP	32	++	No cirrhosis				Alive
38	♀	NP	31	++					Barbiturate and alcohol intoxication
34	♂	NP	26	+					Heart failure
43	♀	P	23	++	No cirrhosis				Alc.
56	♀	NP	21	++					Coronary infarction
54	♂	NP	21	+	No cirrhosis				Pyelonephritis
37	♂	P	21	+	No cirrhosis				Coronary infarct
57	♂	P	16	++					Heart failure
44	♂	P	15	++	No cirrhosis				Alc.
72	♂	P	13	++	No cirrhosis				Alc.
36	♂	P	12	++	No cirrhosis				Melena, no cause indicated
51	♂	P	11	+	No cirrhosis				Cerebral hemorrhage
47	♂	P	11	+					Hypoxic intoxication
33	♂	P	10	++	N cirrhosis				Suffocation
38	♀	NP	10	+++					Arteriosclerotic heart disease
31	♂	P	9	+	No cirrhosis				Alcoholic intoxication
38	♂	NP	9	+	No cirrhosis				Heart failure
43	♀	NP	9	+	No cirrhosis				Cerebral hemorrhage
74	♂	P	9	++					Heart failure
51	♂	P	8	++					Heart failure
49	♂	P	6	++	Steatosis hepatidis				Cerebral hemorrhage
67	♂	P	5	+++	N cirrhosis				Arteriosclerotic heart disease
30	♂	NP	4	++					Cancer of the stomach
54	♂	P	4	++					Alcoholic intoxication
44	♂	P	3	+					Heart failure
41	♂	P	3	+					Heart failure
39	♀	NP	2	+++	No cirrhosis				Uremia
36	♂	NP	2	++	Cirrhosis?				Hepatic coma
77	♀	NP	9/12	+					Heart failure
62	♀	NP	5/12	++	No cirrhosis				Cancer of the gallbladder
58	♂	NP	5/12	+					Cancer of the stomach
68	♂	NP	5/12	++	No cirrhosis				Proteinuria
53	♂	P	2/12	++	No cirrhosis				Cancer of the liver
51	♂	NP	1/12	++	N cirrhosis				Cancer of the stomach
56	♂	NP	1/12	++	No cirrhosis				Lymphoplasmatocytosis
53	♂	NP	1/12	++	No cirrhosis				Gall stones
51	♀	NP	1/12	++	No cirrhosis				Cancer of the spleen
71	♀	NP	1/12	++					Heart failure
49	♂	P	1/12	+++					Cancer of the stomach
70	♀	NP	1/12	+++	N cirrhosis				Cancer of the gall bladder
34	♂	P (emigrat.)	—	++	—	—	—	—	—

NP not present, P = present.

43 patients various degrees of steatosis were still present.

Table II gives some information about 41 patients with an observation period of up to 32 years (median 7) but with no histological examination at follow-up. Information of the latest diagnosis of the liver disease was based on data

available from autopsy (19 patients), death certificate (17 not autopsied patients) and/or clinical observations. Because of emigration no follow-up information was available in one of the 41 patients listed in the Table.

None of the 11 alcoholics in this group developed signs or symptoms of cirrhosis of the liver

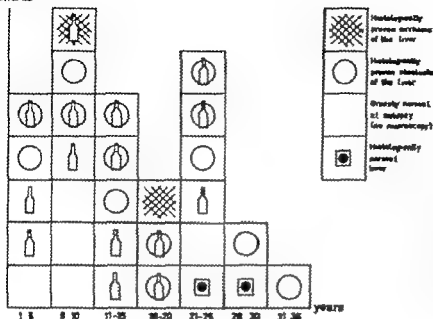
number of
patients

Fig. 2. Results of follow-up examination of patients with duration of observation of more than one year (Patients examined by repeated biopsy or autopsy only). The signs with bottle heads patients with chronic alcoholism.

In one case steatosis was found macroscopically at autopsy.

Of the 23 non-alcoholic patients one died from hepatic coma and probably had cirrhosis, but no autopsy was made. In none of the other patients cirrhosis of the liver was suspected.

Fig. 2 shows the result of the follow-up examination in the 29 patients in whom a definite diagnosis as to the presence or absence of cirrhosis could be established by biopsy or autopsy and in whom the duration of observation was more than one year. Cirrhosis of the liver was found in one of 15 chronic alcoholics and in one of 14 non-alcoholic patients in this group.

DISCUSSION

It is still an open question whether alcoholic steatosis is a step in the development of cirrhosis of the liver or whether alcoholic steatosis and alcoholic cirrhosis are two different modes of reaction in alcoholics.

The aim of the present study was to attack this problem by examining the long-term prognosis in patients suffering from hepatic steatosis, and by studying the development of alcoholic steatosis in particular. If alcoholic steatosis is a state in the development of cirrhosis of the liver one would expect, during a sufficiently long pe-

riod of observation, to find a number of cirrhotics in the group of patients suffering from steatosis due to alcoholic abuse. The most striking result of the present study was, however, that nearly all of the patients, both with and without alcoholic abuse retained a certain degree of fatty liver during the years. In the group of patients with alcoholic steatosis only one of 28 patients developed cirrhosis of the liver (i.e. one of 16, in whom the condition of the liver was verified by autopsy or repeated biopsy).

Also in 30 patients with non-alcoholic steatosis a strikingly high frequency of persisting fatty liver was found.

Cirrhosis was found only in one (suspected in two) of the 21 patients in whom the diagnosis was verified by autopsy or repeated biopsy.

Although it has been confirmed statistically that a certain correlation exists between alcoholic abuse and development of steatosis and cirrhosis (1) this need not be synonymous with a causal relationship between the two. Our results rather support the hypothesis that steatosis and cirrhosis may represent two different responses from the liver. This means that also other factors than abuse of alcohol must be of importance in the development of cirrhosis.

The present study was a retrospective one and consequently subject to a number of errors, as

some of the information required was lacking in the available material. The advantage of the study is the long duration of observation. Therefore, the rare occurrence of cirrhosis in the group of alcoholics may be significant. A prospective study of an unselected material of chronic alcoholics followed over many years might possibly throw some light on the problem. What are the factors which influence the development of alcoholic cirrhosis?

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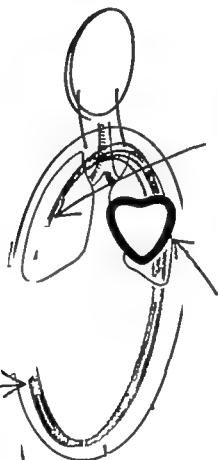
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THE DIAGNOSTIC VALUE OF THE INHIBITORY EFFECT OF ETHANOL ON GALACTOSE ELIMINATION IN LIVER DISEASE

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Abstract. The inhibitory effect of ethanol on the galactose elimination as calculated from changes in the galactose elimination capacity and the galactose half-time was examined in three normal individuals, four patients with cirrhosis and two patients with steatosis, most of whom were alcoholics. The degree of ethanol inhibition did not differ in patients with cirrhosis from that in alcoholic patients with steatosis. According to these results the ethanol inhibition of the galactose elimination is not a specific diagnostic test for steatosis. The galactose elimination capacity during ethanol administration was of the same order of magnitude in the different groups of patients with liver disease as well as in the normal individuals. The cause of this phenomenon and the reason why the inhibition of ethanol is smaller in liver disease remains to be elucidated.

It has been shown previously that ethanol inhibits the galactose elimination in man (1, 12, 18). This effect has been confirmed by studies on rat liver homogenates (5).

In 1961 Tygstrup and Lundquist (17) showed that ethanol inhibited the galactose elimination significantly less in patients with cirrhosis of the liver than in normals, as calculated from changes in the galactose elimination capacity (GE). In 1967 Salaspuuro (10) demonstrated that ethanol inhibited the galactose elimination less in patients with alcoholic steatosis than in normals, as determined from changes in the galactose half-time ($T/2$) and suggested that this fact could be used for the early diagnosis of steatosis in human alcoholics.

The purpose of our work was to examine the effect of ethanol on the galactose elimination in normals, in patients with steatosis and in patients with cirrhosis of the liver in order to evaluate the diagnostic possibilities of the combined ethanol-galactose tolerance test.

MATERIAL

Seventeen patients were examined (Table 1). By questioning patients as well as retrieving the daily consumption of ethanol and the composition of their diet was obtained. In all patients biopsy of the liver was performed before the examination. From the histological diagnosis the patients were grouped as follows: normals, steatosis, and cirrhosis. The liver biopsies were examined by Professor H. Poulsen.

According to the daily ethanol consumption the steatosis group was divided into alcoholic and non-alcoholic steatosis. The group with normal liver histology included three patients with clinical diagnosis of chronic bronchitis, gastritis, and anxiety neurosis, respectively. One of these, formerly consuming more than the equivalent of 120 g 100% ethanol/day for several years, had been abstinent for the last three months; one had consumed more than 120 g/day during several years; and one was occasionally consuming unknown amounts of ethanol. The non-alcoholic steatosis group included three patients, none of whom had consumed more than 30 g ethanol/day; one had a malignant tumour of the lung, one was obese, and one suffered from loss of weight for unknown reasons. The alcoholic steatosis group included seven patients all consuming between 120 and 300 g ethanol/day. None of these patients had other diseases known to cause steatosis. The cirrhosis group included four patients. In two the cirrhosis was probably caused by chronic alcoholism, and two were posthepatic. In one patient with alcoholic cirrhosis (no. 14) the histological examination revealed severe steatosis as well as cirrhosis.

None of the patients had fluid retention. There was no anamnestic evidence of protein- or vitamin-insufficient diet in any of the patients.

METHODS

The patients were examined after fasting for 14 hours. The examination was performed with the patient in the supine position. A typical examination procedure is shown in Fig. 1. At each examination 2.78 mmol (0.5 g) galactose/kg b.wt. was given intravenously for 3 min, and

Table I. Patients grouped according to the histological diagnosis and the daily alcohol consumption

Groups	Pat. no.	Sex	Age (y.)	Histological diagnosis	Daily alcohol consumption (100% ethanol, g)
Normals	1	♂	58	Normal liver	0
	2	♂	57	Normal liver	> 120
	3	♂	31	Normal liver	Unknown
Non-alcoholic steatosis	4	♂	65	Steatosis	0
	5	♀	67	Steatosis	II
	6	♀	37	Steatosis	30
Alcoholic steatosis	7	♂	40	Steatosis	> 120
	8	♀	54	Steatosis	200-300
	9	♂	48	Steatosis	> 120
	10	♂	44	Steatosis	175-200
	11	♂	52	Steatosis	> 120
	12	♂	58	Steatosis	> 120
	13	♂	69	Steatosis	> 120
Cirrhosis	14	♀	67	Cirrhosis	0
	15	♀	47	Cirrhosis	0
	16	♂	50	Cirrhosis, steatosis	> 120
	17	♀	64	Cirrhosis	> 90

= slight, = medium, = severe.

arterial blood concentration was measured at 5-min intervals for 90 min. The sampling technique has been described previously. At the end of the first galactose tolerance test, ethanol was given intravenously in isotonic saline, first as a bolus injection for 8-18 min (mean 1.0 mmol/kg b.w.t. \pm 0.5) followed by a continuous infusion (mean 0.5 g b.w.t., S.D. 0.7) for 102-112 min. The doses were calculated from the average normal ethanol elimination rate of the patients. Infusion as well as bolus injection were done with calibrated pumps. During the test, which started half

an hour after the end of the first, i.e. 30 min from the beginning of the ethanol administration, arterial blood for ethanol determination was also taken at 10-min intervals. The ethanol analyses were performed by modified alcohol dehydrogenase method. Average ethanol concentrations will be seen in Table II.

Urine was collected during 3 h following the study and the amount of galactose was taken to have been excreted during the experimental period. Blood and urine were analysed for galactose by means of galactose oxidase method (7). GIE was calculated according to Tytgstrup (14) (Fig. 2).

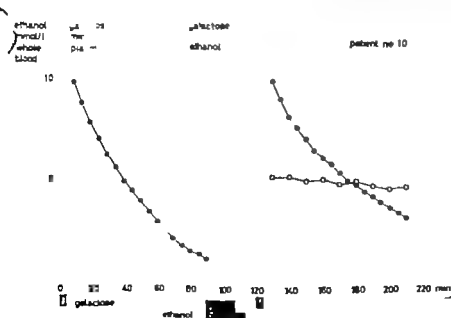


Fig. 1 Examination procedure.

Table II The effect of ethanol on galactose elimination

Groups	Pat. no	GE (mmol/min)		Mean ethanol concentration \pm SD (mmol/l whole blood)
		Control period (I)	Ethanol period (II)	
Normals	1	2.5	1.7	0.7
	2	4.0	1.6	0.4
	3	2.3	1.0	0.4
Mean		2.9	1.4	0.5
Non-alcoholic steatosis	4	1.6	1.5	0.9
	5	2.4	1.0	0.4
	6	2.2	0.9	0.4
Mean		2.1	1.1	0.6
Alcoholic steatosis	7	1.9	1.7	0.9
	8	1.6	0.8	0.5
	9	1.8	1.4	0.8
	10	1.4	0.9	0.7
	11	1.9	1.7	0.9
	12	2.2	1.9	0.9
Cirrhosis	13	2.8	2.3	0.8
	14	1.2	0.9	0.8
	15	1.0	0.9	0.9
Mean	16	3.0	2.3	0.8
	17	1.0	0.7	0.7
Mean		1.6	1.2	0.8

RESULTS

The results are shown in Table II. The group of normals had a GE within normal range (>2 mmol/min) (15). In the non-alcoholic steatosis group one patient had an abnormal and the other two a normal GE. In the alcoholic steatosis group five patients had an abnormal, the other two a normal GE, and in the cirrhosis group three had an abnormal and one a normal GE.

The inhibitory effect of ethanol on galactose elimination, as determined from the relation between GE during and before ethanol administration, was significantly greater in normals than in patients with alcoholic steatosis ($p < 0.05$, t -test) and with cirrhosis ($p < 0.05$). The inhibition in the non-alcoholic steatosis group did not differ from any of the other groups. There was no difference in the degree of inhibition between patients with alcoholic steatosis and those with cirrhosis ($p > 0.8$).

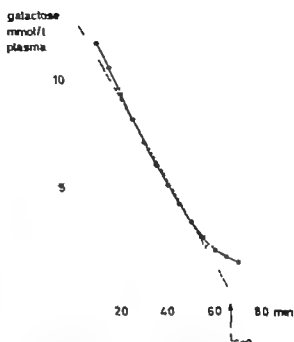


Fig. 2. Galactose elimination capacity (GE) calculated from $GE = M - U / (t_{0-8} + 7)$, where M is amount injected, U amount excreted in urine, t_{0-8} time to zero concentration calculated from the slope of the elimination curve in the interval 20 min after the injection to concentration of 2.22 mmol/l plasma. 7 min is the empirically found correction for uneven distribution of galactose.

DISCUSSION

The elimination of galactose from the blood takes place mainly in the liver where galactose is converted to glucose. The enzyme UDP-galactose-4-epimerase is known to require the presence of NAD and to be inhibited by $NADH_2$ (8). Examinations on rat liver homogenates (5) show that the galactose oxidation is inhibited by the presence of ethanol because of the increase in $NADH_2/NAD$ ratio caused by the metabolic conversion of ethanol.

The inhibition of the galactose elimination by ethanol in man is maximal at plasma concentrations of ethanol above 2.2 mmol/l (17). In 14 of the patients examined in our material the concentration of ethanol was above this value during the whole procedure, whereas in three patients (nos. 1, 7, 9) it was above during the first and a little below during the second half of the period (Table II).

The mechanism whereby ethanol inhibits the

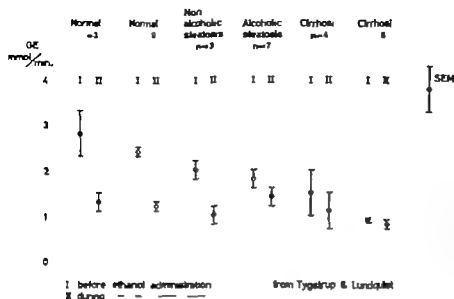


Fig. 3. Galactose elimination capacity (GE) before and during ethanol administration. Data from the present study and from Tygstrup and Lundquist (17).

galactose elimination more in normals than in steatosis and cirrhosis patients is still hypothetical. In choline-depleted rats with fatty liver Salsapuro (11) showed that the galactose elimination was not inhibited by ethanol and concluded (10) that the fatty liver has an increased capacity to oxidate the cytoplasmatic NADH_2 or that the control with the cytoplasmatic redox potential in this condition is different. One study (19) shows that the NADH_2/NAD ratio estimated by the lactate/pyruvate ratio in the hepatic veins is smaller in patients with cirrhosis than in normals during ethanol load, indicating that the shuttle mechanism is functioning relatively well in the cirrhotic liver or that another mechanism is active in the transport of reducing equivalents into the mitochondria.

Another possibility might be that the extrahepatic elimination of galactose is larger than presumed previously. If the extrahepatic conversion of galactose is of the same order of magnitude in normals as in patients with cirrhosis, the effect of ethanol on the total elimination will be relatively smaller in patients with cirrhosis. If it is assumed that only the hepatic removal mechanism of galactose is affected by ethanol, it is remarkable that the GE during ethanol administration is of the same order of magnitude in normals, in steatosis and in cirrhosis patients (mean 1.3 mmol/min, S.D. 0.5) (Table II). Provided that ethanol inhibits the hepatic galactose elimination 100% this would correspond to an

extrahepatic elimination of about 50% which is far above the level previously reported (16). The question regarding extrahepatic galactose elimination needs further investigation, but in this connection it might be of interest that patients in terminal hepatic coma (9) with probably minimal liver function have a GE only a little lower (0.8 mmol/l, S.D. 0.1) than we found during ethanol administration.

As a further hypothetical possibility galactose could be eliminated in the liver in other ways not affected by the increase in NADH_2/NAD ratio related to the ethanol elimination. That galactose can be eliminated by other pathways was shown by Cuatrecasas and Segal (2), who found in experiments on rat liver homogenates that galactose can be converted by galactose dehydrogenase to galactonolactone by the presence of NAD^+ but this step, too, is inhibited by an increased NADH_2/NAD ratio (3). The results of our investigation provide no means to decide which of the above possibilities is correct.

As the number of patients in some of our groups is small, we have included the material from Tygstrup and Lundquist (17). Their material comprising eight normals and five patients with cirrhosis of the liver was examined by a similar technique to that used in the present study. From their experimental data GE has been calculated as mentioned above (Fig. 2). The total material is shown in Fig. 3.

The relative inhibitory effect of ethanol on

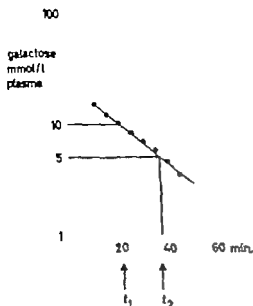


Fig. 4. Galactose half-time ($T/2$) calculated from semi-logarithmic plot of the entire elimination curve, $T/2 = t_2 - t_1$.

the GE is again found to be significantly greater in normals than in patients with alcoholic steatosis ($p < 0.001$, t -test) and with cirrhosis ($p < 0.001$). There is no difference in the degree of inhibition between alcoholic steatosis and cirrhotic patients ($p > 0.6$).

By analysis of variance it is found that there are statistical differences in the values of GE in the different groups in the control period ($p < 0.001$), while this is not the case during the ethanol period ($0.2 < p < 0.6$).

There are two methods for calculating the galactose elimination from blood. By one method (Tygstrup (14)) (Fig. 2) a linear part of the blood elimination curve (zero order kinetics) is used for calculating the GE, and by the other (Tengström (13)) the whole blood elimination curve plotted in semilogarithmic system (first order kinetics) is used for calculating the galactose $T/2$ (Fig. 4).

So far it has not been made clear which of the two methods is the more physiological for expressing the galactose elimination. Therefore we calculated the galactose $T/2$ in the whole material (Fig. 5), well knowing that the injected amount of galactose was 43% more than used by Tengström. We found that the inhibitory effect of ethanol, determined as the relation between the galactose $T/2$ during and before ethanol administration, was significantly greater in normals than in patients with alcoholic steatosis ($p < 0.05$) and on the borderline of significance in relation to patients with cirrhosis ($0.05 < p < 0.1$), while there was no difference between the inhibition in alcoholic steatosis and cirrhotic patients ($p > 0.6$).

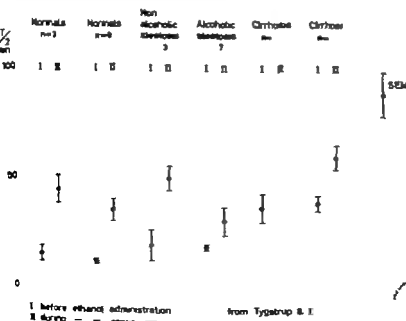


Fig. 5. Galactose half-time ($T/2$) before and during ethanol administration. Data from the present study and from Tygstrup and I.

The corresponding analysis of variance cannot be carried out on the T/2 values from the control period, as the groups are not statistically comparable, but during the ethanol period the difference between the groups is significant ($0.01 < p < 0.02$) contrary to the finding for GE.

Our investigations confirm that the inhibitory effect of ethanol on the galactose elimination, calculated from changes in GB as well as T/2, is greater in normals than in alcoholic steatosis and cirrhosis patients, but the alcoholic steatosis group has the same degree of inhibition as the cirrhosis group. Therefore the ethanol inhibition of galactose elimination has no differential diagnostic value in hepatology. It must be assumed that the degree of liver damage rather than the specificity of the histological abnormality is responsible for the changes in galactose elimination caused by ethanol.

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ISOENZYME PATTERNS OF SERUM ALKALINE PHOSPHATASE IN ETHANOL-INDUCED LIVER INJURY

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Abstract. Abnormal agar gel electrophoretic patterns of alkaline phosphatase (AP) isoenzymes have been demonstrated in 11 out of 16 chronic alcoholics in spite of normal or borderline routine liver tests (total AP, GOT, GPT, bilirubin). The abnormality consisted of the presence of fast-moving α_2 -fractions known to be related to hepatic disease preferably of obstructive type. The presence of α_2 -AP appears to be more reliable indicator of ethanol-induced liver damage than LDH 5 and OCT.

In a recent study (1) of various enzymes connected with liver injury abnormal isoenzyme patterns of alkaline phosphatase (AP) were often noted in 18-year-old boys who were examined in connection with enlistment for compulsory military service. The abnormality consisted in demonstrable fast moving fractions (α AP) on agar gel electrophoresis, which finding is usually related to significant hepatobiliary disease (15-25). It appeared possible that α_1 AP was related to alcohol damage. Abuse of alcohol has been reported to increase at this age in Sweden as well as in other countries (2, 8, 19).

The aim of the present study was to find out whether or not α_1 -AP might be a sensitive indicator of alcohol damage to the liver. For this purpose analyses were made on a material of known alcohol abusers presenting values within the normal range for routine liver tests (transaminases, bilirubin, total AP).

In addition to α_1 -AP lactate dehydrogenase (LDH) isoenzymes and ornithine carbamoyl transferase (OCT) were included for comparison, since these tests are known to be sensitive indicators of ethanol-induced liver damage (4, 11, 14).

MATERIAL

The study was performed on chronic alcoholics treated in the Medical Department of Södersjukhuset, Stockholm. From this material 16 males presenting normal or borderline values for the routine liver tests (transaminases, bilirubin, AP) were selected for further studies. Their ages ranged between 35 and 60 and all of them displayed several years' history of alcoholic abuse.

Comparative studies were also performed on 118 blood donors, medical students and healthy members of the hospital staff.

METHODS

For analysis of the following variables venous punctures were made in the fasting state in the morning.

Transaminases (GOT and GPT) and lactate dehydrogenase (total LDH and LDH isoenzymes) were assayed on the day on which the samples were drawn. Ornithine carbamoyl transferase (OCT) and isoenzymes of AP were analysed on serum samples stored at -20°C .

LDH isoenzymes were assayed by an automatic fluorometric method standardized by kinetic UV method (9). Normal values 5-17 U/l (borderline values 17-20).

LDH isoenzymes were measured by the agar gel electrophoretic method of Wrensch (24) with slight modifications (21). By this method mean LDH 5/LDH 4 ratio of 0.75 ± 0.02 (mean \pm S.E.M.) was found for 62 healthy persons (laboratory staff, medical students). A LDH 5/LDH 4 ratio exceeding 1.0, found in only 4 of the 62 above mentioned persons, is in the present study taken as indicative of liver injury.

The OCT activity was determined by incubating serum with citrulline carbamoyl ^{14}C in arsenate buffer (16). The results are expressed in $\mu\text{moles } ^{14}\text{CO}_2$ liberated by 10 ml serum in 1 min incubation under standard conditions. Normal values <0.24 U/l (7).

Total AP activity was measured by an AutoAnalyzer procedure (17) standardized by the manual method of

Table I. Serum activity of liver enzymes in 16 alcoholics

	Total AP	α_1 -AP	LDH 5/LDH-4 ratio	OCT	GOT	GPT
Pathological values	1	11	7	3	2	0
Borderline values	2	2	1	1	1	2
Normal values	13	3	8	12	13	14

Laurent and Norberg (12). Normal values 20-85 U/l (borderline values 85-105).

AP isoenzymes were measured by a modification (22) of the agar gel electrophoretic method of Wlasek (24) using the staining technique described by Tarwell and Jeffers (23). An α_1 -AP fraction of more than 3 U/l is considered to be pathological and a demonstrable fraction below 3 U/l (trace) is considered as probably pathological. Of 118 blood donors, medical students and healthy members of the hospital staff distinct α_1 -AP fractions were found in only 2 cases and trace amounts in 7 cases. Five of these cases also displayed pathological LDH 5/LDH-4 ratios.

RESULTS

Fig. 1 shows AP isoenzyme patterns in 3 alcoholics as compared with the normal pattern and the pattern of severe hepatic disease.

Table I summarizes results in 16 alcoholics with normal or nearly normal total AP, GOT and GPT. Distinct α_1 -AP fractions were found in 11 cases and trace amounts in 2. In contrast, pathological AP, LDH 5 and OCT were only found in 1, 7 and 3 cases, respectively. The α_1 fraction averaged 9.4 U/l in the 11 cases and constituted -24% (mean 13%) of the total AP activity.

DISCUSSION

A fast-moving α_1 fraction of serum alkaline phosphatase may be demonstrated in certain pathological sera by a variety of electrophoretic techniques. Several reports are available showing the relation between the occurrence of α_1 -AP and hepatobiliary disease, especially of intra or extra-hepatic obstructive type (7, 10, 13, 18, 22 and others). The presence of α_1 -AP in spite of normal total AP has been suggested to be of clinical diagnostic importance since the fraction may be the only abnormal clinical chemical finding in neoplastic hepatic disease (7, 13). The finding here that chronic alcoholics very often show distinct α_1 fractions tends to lessen the diagnostic value of at least, small and moderate fractions.

The value of the test may on the other hand, prove to be that it is a very sensitive aid in the diagnosis of alcoholic abuse. It is apparent from

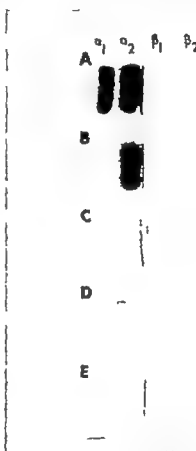


Fig. 1. Agar gel electrophoresis of serum AP. A shows α_1 and α_2 -AP in a case of hepatic cancer. The thin line immediately cathodal to the α_2 fraction is the site of serum application. B shows α_1 and α_2 -AP in an alcoholic with slightly elevated transaminases (GOT 21, GPT 21 U/l). Total AP = 94 U/l. C = trace α_1 -AP but otherwise normal findings in an alcoholic with normal transaminases (GOT 15, GPT 13 U/l). Total AP = 104 U/l. β_1 = the bone fraction, β_2 = the foetal AP (22). D shows a distinct α_1 fraction in an alcoholic in spite of low transaminase values (GOT 6, GPT 4 U/l) and low total AP (66 U/l). E shows absence of activity in the α_1 region in a normal person (Total AP 59 U/l).

our data that there are stages of ethanol-induced liver involvement when α_1 AP and LDH 5 are increased in serum, in spite of normal values for transaminases and OCT. The explanation is hardly differences in rate of elimination of the enzymes from serum, since these rates are similar for OCT and LDH 5 (5, 6).

The occurrence of α_1 -AP in serum is significantly correlated to an increased LDH 5/LDH-4 ratio both in clinical material (22) and in clinically healthy young men (1). However the general opinion is that the fraction is not directly related to liver cell damage but to regurgitation into the sinusoids of bile isoenzyme in cases with obstruction (15). It appears likely that also in alcoholics α_1 -AP reflects a cholestatic component of the liver involvement (20).

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LIVER LIPID CONTENT IN ALCOHOLICS

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Abstract. Liver biopsy specimens from 51 alcoholics obtained with 0.7 mm needle have been analysed for lipid content and these values have been compared with the evaluation of visible fat in smears from the same biopsy specimens. Large variations in liver lipid concentrations have been found, ranging from one group, 24% of total, with fat within normal limits to a few individuals with 100-fold increase in liver lipid concentration. Partly this variation is explained by varying abstinence time before biopsy. A classification of liver lipid levels into three morphological categories is suggested and will give an adequate measure of this parameter. Serum transaminases and bilirubin were the only laboratory data found to co-vary with liver fat.

Chronic intake of alcohol leads to hepatic steatosis of a variable degree. The quantitative extent and variation of this liver fat accumulation has previously been measured in only a few studies (3-5). Recently methods for the chemical determination of lipids in liver specimens obtained by the fine-needle aspiration biopsy technique have been developed (1, 3-10). By these methods quantitation of liver lipids can be performed without risk and with little inconvenience to the subject (6).

In the present study the liver fat has been determined in a series of 51 alcoholics admitted to a Swedish University Hospital. A comparison has been made between these data and estimation of visible fat in the liver cells. Some relevant laboratory data have also been related to the liver lipid values.

MATERIAL

The subjects are alcoholics admitted to the University Hospital of Lund. They were classified as alcoholics with the following two criteria: they had pathological desire for alcohol after ingestion of small doses and showed physical dependence on alcohol after withdrawal. All cases

with verified liver cirrhosis or other accompanying diseases, e.g. diabetes mellitus or extreme obesity (18 individuals), have been excluded and the final material consisted of 51 cases (49 men, 2 women), aged 48 ± 11 years (mean \pm S.D.). Liver lipids were determined in 9 healthy controls (5 men, 4 women), aged 50 ± 14 (mean \pm S.D.) described elsewhere (11). From this control material the upper "normal" limit was determined as mean of triglyceride concentration $+2$ S.D.

METHODS

Liver biopsy with 0.7 mm needle was performed as described previously (7) (11 ± 7 days (mean \pm S.D.) after admission to hospital, or after an overnight fast in controls). Cytological examination (7) and chemical determination of liver lipids (1-10) were performed on parts of the same biopsy specimen.

Morphologically liver fat was scored from 0 to 3 (7): 0 = no or only few small vacuoles, 1 = small vacuoles in minority of the cells, 2 = small or large vacuoles in majority of the cells, 3 = small and/or large vacuoles in almost every hepatocyte.

Liver triglyceride concentration, chemically determined, was expressed as molar ratio triglycerides over phospholipids (TG/PL) (1, 10). Only triglycerides accumulate in alcoholic fatty liver and phospholipids therefore constitute suitable reference substance (2, 3-10). Conventional biochemical liver function tests have been performed at the Department of Clinical Chemistry University Hospital of Lund, according to routine procedures. These data were retrospectively collected from the hospital records. These tests had been performed one or two days after admission to the hospital. The coefficients of correlation were tested against zero by assuming normal populations; S.D. always expresses standard deviation of sample.

RESULTS

Liver lipid contents. As shown in Fig. 1 there was a great variation in individual liver lipid values. The majority of the cases had TG/PL ratio of more than 2.0. Since human liver phospholipid concentration is approximately 35 μ moles/g wet

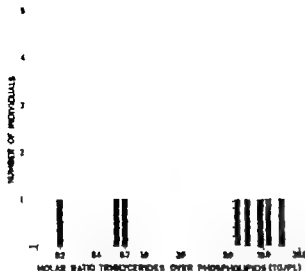


Fig. 1. Liver triglyceride accumulation in 51 alcohol abusers. Mean ± 2 S.D. of control material was 0.9.

weight (10), this value would correspond to a triglyceride concentration of 6% of liver wet weight. The lowest values correspond to a triglyceride concentration of 0.6% while, at the other extreme, half of the liver mass would consist of triglycerides—a variation of almost two orders of magnitude.

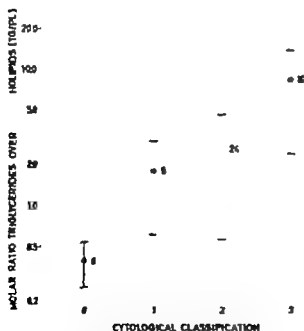


Fig. 2. Liver triglyceride concentration expressed as the molar ratio TG/PL within each of the scores of fatty vacuolation (mean \pm S.D.). The figures beside the bars are the numbers of individuals in each group.

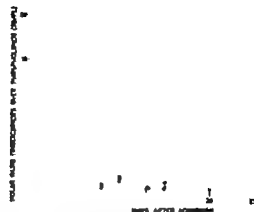


Fig. 3. Liver triglyceride concentration expressed as the molar ratio TG/PL compared with the time after admission before liver sampling.

The control material had a TG/PL ratio of 0.34 ± 0.26 (mean \pm S.D.). Twelve individuals (24% of the total number of alcoholics) had TG/PL ratios below 0.9 (mean ± 2 S.D. for control material) and were thus considered as having a normal liver lipid content.

Cytological estimation. Fig. 2 shows the scores of fatty vacuolation of liver fat plotted against the chemically determined values. A rough correlation was obtained with a great deal of overlap between categories. Category 1 did not differ significantly from category 2, but the differences between categories 0 and 1, 1 and 2, and 2 and 3 were all statistically significant ($p < 0.01$). The lower limit for cytologically detectable liver fat corresponds to a TG/PL ratio of approximately 0.7 (mean ± 2 S.D. for the 0 category). The liver lipid values within the 0 category did not differ statistically from the control material.

Factors affecting the liver lipids. In view of the very marked individual differences in liver lipid content (Fig. 1) some possible causative factors were studied. Age was not correlated with liver lipid concentrations. The number of days from admission to the day of liver biopsy (11 ± 7 (mean \pm S.D.)) was not significantly correlated with liver lipid values, the correlation coefficient being 0.035 (Fig. 3). In 34 cases data of abstinence time before liver biopsy were obtained and found to be 13 ± 8 (mean \pm S.D.). These values gave a weak negative correlation between the molar ratio TG/PL and abstinence time, the correlation coefficient being -0.34 ($0.01 < p < 0.05$) (Fig. 4).

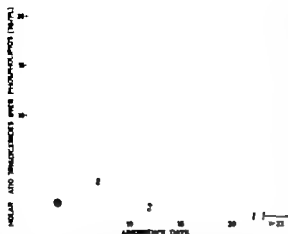


Fig. 4 Liver triglyceride concentration expressed as the molar ratio TG/PL compared with alcohol abstinence time before liver biopsy. O—the patient described in the Discussion.

Retrospective studies of the records of the patients did not give any information of amount and duration of daily alcohol intake or of dietary pattern.

Correlation of liver lipids with other laboratory data. Small to moderate increases in serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum bilirubin (bi/s) were seen in several cases. The correlation coefficients between these parameters and the liver lipid values were 0.53, 0.33 and 0.60, which are significantly different from zero ($p < 0.001$, $0.01 < p < 0.05$ and $p < 0.001$ respectively). ESR, serum alkaline phosphatase, serum lactate dehydrogenase (LDH) and bromsulphthalein (BSP) retention test were not significantly correlated to the level of liver lipids.

DISCUSSION

The most striking finding of the present study was the large individual variation in degree of liver fat accumulation. Almost 1/4 of the material had liver lipid concentrations within normal limits. On the other hand, the majority of the cases had a moderate to severe hepatic steatosis with lipids amounting to 6–50% of liver wet weight.

As expected, one explanation of this variation was the duration of abstinence time before the biopsy. However, this factor alone does not seem

to explain the highly varying degree of liver lipid concentration. Even with a verified short abstinence time some individuals had much lower liver lipid values than others. For example, as illustrated in Fig. 4, one subject had a molar TG/PL ratio of 1.3 in spite of heavy drinking (150 g ethanol/day) up to 3 days before biopsy. This patient had been drinking the same daily amount for at least 15 years. On admission he had a bi/s of 0.9 mg%, SGOT of 53 U, SGPT of 90 U and a BSP retention time after 30 min of 4%. It is probable that some of the cases with low lipid values at short times after admission in Fig. 3 illustrate the same thing, but it has not been possible to obtain their exact abstinence times. The present study does not exclude differences between individuals in amount and duration of alcohol intake or dietary pattern, and such factors are important (5).

Large differences between individuals concerning hepatic lipid accumulation after ethanol intake have been observed previously. Thus, in other studies a group of alcoholics (1/3–1/4 of total) has been found which showed no histologically visible liver fat (4, 9). Upparte et al. (9) failed to bring about liver fat accumulation in such individuals on administration of large amounts of alcohol for 10–12 days, although fat infiltration readily reappeared in the remaining 75% of the alcoholics. This was taken as an indication of constitutional differences in the individual response to the effects of alcohol metabolism. It is possible that the finding of some cases in this study with normal or almost normal hepatic lipid concentrations after a short time of abstinence may indicate a similar mechanism.

In view of the comparison between cytological estimation and chemical liver lipid quantitation it does not seem justified to classify liver fat in fine-needle biopsy specimens into more than three categories. For routine purposes this would allow a fairly adequate estimation of liver lipid levels. The lower limit for detectable fat by this method would be approximately 2–3% of the wet weight, similar to that found by others (2) using a histological technique.

ACKNOWLEDGEMENTS

Financial support was obtained from Stiftelsen Svensk Narkotikaforskning, Uppsala, and P. Håkansson Stiftelse, Fåfö, Sweden.

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TRACE ELEMENTS IN SERUM AND URINE FROM HYPERTENSIVE PATIENTS BEFORE AND DURING TREATMENT WITH CHLORTHALIDONE

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Abstract. Urinary excretion of antimony, arsenic, bromine, cadmium, caesium, cobalt, copper, gold, iron, mercury, molybdenum, rubidium, scandium, silver, tungsten and zinc from 16 hypertensive patients before and during treatment with chlorthalidone has been investigated. The serum concentration of these elements before and after treatment was determined in 10 of the patients. The method consisted of neutron activation analysis combined with recently developed ion exchange technique. The serum concentration as well as the urinary excretion of the 17 trace elements investigated in pretreated hypertensive patients did not differ significantly from those of normotensive subjects. During treatment with chlorthalidone, raised urinary excretion of bromine, cadmium, cobalt, rubidium and zinc and decreased urinary excretion of caesium were observed. A significantly decreased serum concentration after 5 days treatment was only noted for rubidium.

A possible connection between trace elements and elevated blood pressure was indicated by the observation that experimentally induced hypertension in rats could be temporarily corrected by the administration of EDTA (22). Intravenous administration of CaNa_2EDTA to hypercholesterolaemic patients has been reported to lower the serum cholesterol and at the same time to produce a more than tenfold increase in the urinary excretion of zinc and smaller increases in cadmium, lead, manganese and vanadium excretion, while the excretion of molybdenum, nickel, silver and tin did not change (14).

Compared to normal urine, that from human hypertensives has been reported to contain increased amounts of cadmium, zinc, manganese, vanadium, lead, nickel and tin (16, 19), the increase in cadmium was especially marked.

Further observations also suggest a possible connection between cadmium and elevated BP. Small amounts of cadmium given to rats have

been reported to cause hypertension (23). Human kidneys contain large amounts of cadmium, as compared to other organs, and the renal concentration of cadmium from various population groups roughly parallels the geographical differences in hypertension (17). However, results of investigations of renal cadmium from hypertensive subjects dying sudden accidental deaths are not in agreement (5, 11) and, furthermore, industrial workers exposed to cadmium did not show an increased incidence of hypertension (6, 27).

It has been asserted that all antihypertensive agents not acting on nerves appear to be chelating agents (20). The effect of chlorthalidone on the urinary excretion of macroelements, i.e. increased sodium and potassium excretion and decreased calcium excretion, is well known, but its effect on trace element excretion does not seem to have been studied.

The aims of the present study were firstly to investigate the serum concentration and urinary excretion of antimony, arsenic, bromine, cadmium, caesium, cobalt, copper, gold, iron, mercury, molybdenum, rubidium, scandium, silver, tungsten and zinc in hypertensive patients and secondly to study the effect of treatment with chlorthalidone on the serum concentration and urinary excretion of these trace elements.

MATERIAL AND METHODS

Sixteen patients with untreated arterial hypertension of varying degree, 11 men and 5 women, ranging in age from 28 to 60 years (mean 46), were subjected to the study. Some data on the patients are presented in Table I. Their diastolic BP on admission varied between 105 and 150 mmHg. Three of the patients (nos. 3, 9 and 12) had pronounced enlargement of the heart and in two

Table I Data on patients subjected to the study

Pat. no.	Age (y)	Sex	BP on admission	Food hypertensive changes	Heart size (ml/m ² BSA)	GFR (ml/min)
1	54	♀	260/190	II	490	71
2	60	♂	180/110	I-II	530	70
3	50	♂	210/120	II	700	140
4	28	♂	175/105	I	400	108
5	40	♀	175/170	I	360	72
6	40	♀	180/110	I-II	370	136
7	58	♂	190/120	II	530	155
8	60	♀	250/120	II	540	49
9	46	♂	190/125	0-I	600	130
10	41	♀	215/115	I-II	400	100
11	29	♂	170/110	I	360	87
12	39	♂	200/130	III	870	140
13	45	♂	240/125	III	510	122
14	56	♂	210/110	I	460	160
15	33	♂	170/105	0-I	370	110
16	33	♂	175/115	0-I	390	60

(nos. 8 and 16) the glomerular filtration rate was reduced below 70 ml/min. The ECG was normal in 13 of the patients. In case 5 the left kidney was reduced in size and there was a compensatory enlargement of the right kidney and in case 8 the right kidney was slightly reduced in size. A small renal artery stenosis on the right side and one minimal stone in each kidney was present in case 12. Bacteruria did not occur in any case. None of the patients had raised amounts of catecholamine in the urine or any other signs of endocrine hypertension.

Urine was collected during two periods of 5 days each, first without any antihypertensive treatment and then

during treatment with chlorthalidone (Hygroton®), 50 mg per os daily. The five 24-hour primary collections from each period were pooled into one sample and 1.2 ml was pipetted into quartz ampoules.

In 10 of the 16 patients (nos. 1, 2, 3, 5, 8, 9, 10, 11, 14 and 15) blood samples were taken in fasting state on the first day of the period without treatment and after 5 days treatment. In cases 11 and 12 blood samples were taken after 3 days treatment and in case 4 solely before treatment.

Blood samples from eight normotensive, apparently healthy individuals in fasting state were also analysed. Five were men and 3 women, ranging in age from 23 to 63 years (mean 43). The blood samples were drawn through plastic catheters inserted in the cubital vein and the first 10 ml of blood were discarded. After centrifugation 1 ml of serum was pipetted into quartz ampoules. All plastic and glass ware used for the collection and preparation of the samples was thoroughly rinsed with 6N HCl and demineralized water. The quartz ampoules were rapidly flame-ashed, as described earlier (3), and irradiated together with standards in the R-2 reactor at Studsvik at a thermal neutron flux of $2 \cdot 10^{16}$ n/cm²/sec for 74 hours. The chemical separation procedure and the γ -spectrometric measurements and identification of the elements have been described elsewhere (18, 30).

Statistical methods

In the statistical analysis the Student's *t*-test has been used when comparing serum concentrations and the paired *t*-test when comparing urinary excretions. The degrees of significance obtained have been expressed as follows: — not significant ($0.05 < p$), almost significant ($0.01 < p < 0.05$), * significant ($0.001 < p < 0.01$), ** highly significant ($p < 0.001$).

Table II 24-hour urinary excretion of trace elements (μ g) with known biological function before and during treatment with chlorthalidone

Case no.	Co		C		F		Zn	
	Before	During	Before	During	Before	During	Before	During
1	0.44	1.9	28	29	51	49	310	320
2	1.8	2.1	37	40	130	81	330	360
3	1.7	1.9	37	61	160	180	460	580
4	0.45	0.94	37	40	59	120	430	730
5	0.67	0.95	4.0	4.0			140	330
6	0.52	0.94	18	57	84	140	280	690
7	0.48	0.74	22	31	110	94	720	1200
8	0.23	1.4	41	61			220	390
9	0.59	0.29	77	51	67	53	800	1400
10	0.72	0.75	48	41	330	97	410	560
11	0.83	0.64	87	81	190	220	290	340
12	0.32	0.41	65	69	140	160	1200	1300
13	0.40	0.32	39	39	100	190	730	830
14	0.52	0.53	86	110	54	180	1000	1200
15	0.63		39	140	220	180	470	720
16	1.4		115	76			500	530
Mean \pm S.D.	0.73 \pm 0.48	0.99 \pm 0.61	50 \pm 29	58 \pm 33	131 \pm 84	134 \pm 56	520 \pm 300	770 \pm 370
Mean difference	+0.34		+8.8		+2.2		+201	

Table III. 24-hour urinary excretion of trace elements (μg) with suspected biological function before and during treatment with chlorthalidone

Case no.	Br		Cd		M		Rb		Se	
	Before	During	Before	During	Before	During	Before	During	Before	During
1	2 500	3 200	1.9	3.9	33	34	1 600	1 900	28	21
2	2 100	3 100	1.8	1.7	89	67	2 000	3 200	29	1
3	3 500	9 900	0.78	1.3	87	110	2 600	4 300	33	37
4	3 700	4 800	0.70	1.0	87	130	3 200	3 900	43	26
5	1 700	2 800	0.54	0.34	34	25	2 000	3 800	16	19
6	4 300	6 600	1.2	4.6	130	120	2 400	5 400	31	41
7	7 000	7 800	0.99	1.2	57	72	3 400	5 300	12	35
8	1 600	2 500	1.2	1.6	51	25	1 300	2 200	14	18
9	3 700	8 900	1.0	2.1	95		3 300	4 500	44	69
10			2.0	2.0	23	21	2 000	2 600	34	41
11	6 700	11 000	2.2	1.7	100	80	2 100	3 900	3	23
12	2 900	4 000	0.24	0.22	56	45	2 000	2 900	49	45
13	3 700	4 200	2.7	2.3	66	134	2 400	4 400	33	32
14	4 300	5 400	8.4	11	180	100	3 100	4 000	38	55
15	2 800	8 000	0.85	1.5	120	240	2 400	5 700	18	30
16	3 500	7 300	6.8	10	80	55			52	49
Mean \pm S.D.	3 700 \pm 1 600	6 000 \pm 2 700	2.1 \pm 2.3	2.9 \pm 3.2	81 \pm 41	84 \pm 58	2 400 \pm 600	3 900 \pm 1 200	30 \pm 14	36 \pm 14
Mean difference	+2 200*		+0.80*		-5.2		-1 600*		-6	

RESULTS

The urinary excretion, expressed as $\mu\text{g}/24$ hours, of the different trace elements studied before and during 5 days treatment with chlorthalidone (Hygroton®) is presented in Tables II-IV. Table II contains some trace elements with known biological function (cobalt, copper, iron and zinc) and Table III some with suspected biological function (bromine, cadmium, molybdenum, rubidium and selenium). Table IV contains the following trace elements without known biological function: antimony, arsenic, caesium, gold, mercury, scandium, silver and tungsten.

The daily urinary excretion from the untreated patients with hypertension did not differ from that of normotensive subjects previously investigated with respect to any of the trace elements studied (1, 2, 3, 32).

Among the trace elements with known biological functions an almost significantly increased urinary excretion was noted for cobalt during, as compared to before treatment with chlorthalidone.

The urinary excretion of zinc was highly significantly raised during, as compared to before, treatment, with a mean difference of 201 $\mu\text{g}/24$ hours.

The excretion of copper and iron did not change significantly.

Among the trace elements with suspected biological function (Table III) a highly significant increase in the excretion of bromine and rubidium and an almost significant increase in the excretion of cadmium were noted during treatment. The urinary excretion of most of the trace elements without known biological function did not change significantly. Caesium, however, showed a highly significantly decreased excretion during treatment.

If the results are expressed in $\mu\text{g/g}$ excreted creatinine per 4 hours, the same statistical correlations are obtained between the trace element excretion before and during treatment.

Tables V-VII contain the trace element concentrations in serum from the 8 normotensive subjects and from the hypertensive patients before and after 5 days' treatment. The values are expressed as mean \pm S.D. The elements have been divided into the same groups as for urine, viz those with known (Table V), suspected (Table VI) and without known biological function (Table VII). The amounts are expressed in $\mu\text{g}/\text{ml}$ for the elements with known and suspected biological function and in $\mu\text{g}/\text{ml}$ for the elements without known biological function.

Table I. Data on patients subjected to the study

Pat. no	Age (y.)	Sex	BP on admission	Funds hyper sensitive changes	Heart size (ml/m ² RSA)	CFR (ml/min)
1	56	♀	260/150	II	430	71
2	60	♂	180/110	I-II	530	70
3	50	♂	210/120	II	700	140
4	28	♂	175/105	I	400	108
5	40	♀	175/120	I	360	72
6	40	♀	180/110	I-II	370	136
7	58	♂	190/120	II	350	155
8	60	♀	250/120	III	340	49
9	46	♂	190/125	0-1	400	130
10	61	♀	215/115	I-II	400	100
11	29	♂	170/110	I	360	87
12	39	♂	200/150	III	870	140
13	48	♂	240/125	III	510	122
14	54	♂	210/110	I	460	160
15	53	♂	170/105	0-1	370	110
16	33	♂	175/115	0-1	390	60

(nos. 8 and 16) the glomerular filtration rate was reduced below 70 ml/min. The I. pyelogram was normal in 13 of the patients. In case 4 the left kidney was reduced in size and there was a compensatory enlargement of the right kidney and in case 8 the right kidney was slightly reduced in size. A small renal artery stenosis on the right side and one infarctal stone in each kidney was present in case 12. Bacteriuria did not occur in any case. None of the patients had raised amounts of catecholamine in the urine or any other signs of endocrine hypertension.

Urine was collected during two periods of 5 days each, first without any antihypertensive treatment and then

during treatment with chlorthalidone (Hygroton®), 50 mg per os daily. The five 24-hour urinary collections from each period were pooled into one sample and 1.2 ml was pipetted into quartz ampoules.

In 10 of the 16 patients (nos. 1, 2, 3, 5, 8, 9, 10, 13, 14 and 15) blood samples were taken in fasting state on the first day of the period without treatment and after 5 days treatment. In cases 11 and 12 blood samples were taken after 5 days treatment and in case 4 solely before treatment.

Blood samples from eight normotensive apparently healthy individuals in fasting state were also analysed. Five were men and 3 women, ranging in age from 23 to 63 years (mean 43). The blood samples were drawn through plastic catheters inserted in the cubital vein and the first 10 ml of blood were discarded. After centrifugation 1 ml of serum was pipetted into quartz ampoules. All plastic and glass were used for the collection and preparation of the samples was thoroughly rinsed with 6N HCl and demineralized water. The quartz ampoules were rapidly flame-sealed, as described earlier (33), and irradiated together with standards in the R-2 reactor at Studsvik at a thermal neutron flux of $2 \cdot 10^{16}$ n/cm²/sec for 24 hours. The chemical separation procedures and the γ -spectrometric measurements and identification of the elements have been described elsewhere (18, 30).

Statistical methods

In the statistical analysis the Student's *t*-test has been used when comparing serum concentrations and the paired *t*-test when comparing urinary excretions. The degrees of significance obtained have been expressed as follows: — not significant ($0.05 < p$), almost significant ($0.01 < p < 0.05$), ** significant ($0.001 < p < 0.01$), *** highly significant ($p < 0.001$).

Table II. 24-hour urinary excretion of trace elements (μ g) with known biological function before and during treatment with chlorthalidone

case no	Co		Ca		Fe		Zn	
	Before	During	Before	During	Before	During	Before	During
1	0.44	1.9	28	29	51	49	310	320
2	1.8	2.1	57	40	130	81	390	360
3	1.7	1.9	57	61	160	180	460	380
4	0.45	0.94	37	40	59	120	430	730
5	0.67	0.95	4.0	4.0			140	330
6	0.52	0.94	16	57	84	140	280	650
7	0.48	0.74	22	31	110	94	720	1700
8	0.23	1.4	41	61			220	390
9	0.59	0.29	77	51	67	53	800	1400
10	0.72	0.73	48	41	350	97	410	560
11	0.83	0.64	87	84	190	220	290	340
12	0.32	0.41	65	69	140	160	1200	1300
13	0.40	0.32	53	39	100	190	770	890
14	0.52	0.53	86	110	54	180	1000	1200
15	0.63		39	140	220	180	470	720
16	1.4		115	76			300	580
Mean \pm S.D.	0.73 \pm 0.48	0.99 \pm 0.61	50 \pm 29	58 \pm 33	131 \pm 84	134 \pm 56	520 \pm 300	720 \pm 370
Mean difference	+0.34		+8.8		+2.2		+201	

Fe		Sb		Se		W	
Before	During	Before	During	Before	During	Before	During
0.96	0.44	2.0	1.1	0.080	0.060	9.7	9.8
1.0	1.0	1.8	2.7	0.011	0.017	8.1	4.1
0.18	0.42	1.4	1.7	0.035	0.036	3.2	15
0.99	0.65	1.2	1.8	0.0039	0.0083	44	49
0.16	0.28	1.2	0.65	0.36	0.34	2.3	5.2
0.61	1.0	0.77	0.97	0.14	0.20	270	360
0.42	0.30	0.52	0.58	0.25	0.43	8.0	13
0.90	1.0	1.5	1.9	0.0079	0.0078	32	25
1.0	2.6	1.2	1.6	0.0039	0.0060	13	46
1.4	1.3	1.0	1.1	0.0060	0.0038	18	6
0.66	0.71	1.7	1.0	0.070	0.030	41	21
1.8	2.6	2.6	2.2	0.024	0.045	12	15
2.4	1.3	2.4	1.2	0.016	0.020	16	2.9
2.4	2.2	1.3	1.4			6.5	19
0.61	0.95	1.2	1.7	0.081	0.027	7.7	7.7
1.4	1.4	2.0				3.0	5.5
1.0 ± 0.09		1.1 ± 0.75		1.3 ± 0.57		1.4 ± 0.58	
+0.009		-0.013		0.073 \pm 0.10		0.097 \pm 0.17	
				+0.024		32 \pm 43	
						+7.4	

day. In the present study 2 patients (nos. 12 and 16) had a mild proteinuria ($\sim 1\%$). Case 16 had the largest amount of copper in urine of all the untreated patients (115 $\mu\text{g/day}$) and in case 12 the copper excretion was 65 $\mu\text{g/day}$ which also is within the limits reported for patients with proteinuria.

The excretion of zinc has also been reported to be high in patients with proteinuria but uncorrelated to the degree of proteinuria (9). Case 12 in the present study had the highest zinc excretion, however close to the mean value, of all untreated patients.

In the following discussion the different trace elements investigated will be grouped under the headings known, suspected or without known biological function, and, where possible, the results will be compared with those of other investigators.

Trace elements with known biological function

Cobalt The cobalt concentration found in serum from healthy subjects is in good agreement with figures for normal concentration presented by other authors (13) and did not differ from that found in the hypertensive patients. The urinary excretion of cobalt from the untreated hypertensive patients does not differ from the figures for normal cobalt excretion presented by Melzer et al. (10) or from those of healthy individuals previously investigated (1, 2, 3, 32). The urinary excretion during treatment with chlorthalidone was almost significantly increased as compared to before treatment, but the serum concentration did not change.

Copper There was no significant difference in urinary copper excretion before and during treatment with chlorthalidone. The mean value is in excellent agreement with the mean urinary ex-

Table V Serum concentration of trace elements ($\mu\text{g/ml}$) with known biological function before and during treatment with chlorthalidone (mean \pm S.D.)

	Patients with hypertension		
	Healthy subjects (n = 8)	Untreated (n = 11)	After 5 days' treatment (n = 12)
C	0.00032 \pm 0.00043 (n = 7)	0.00046 \pm 0.00018 (n = 10)	0.00046 \pm 0.00029
Ca	1.01 \pm 0.33	0.80 \pm 0.27	0.84 \pm 0.29
Fe	1.21 \pm 0.78	1.32 \pm 0.47	0.97 \pm 0.69
Zn	0.93 \pm 0.44	0.96 \pm 0.42	0.96 \pm 0.43

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PROPRANOLOL GIVEN TWICE DAILY IN HYPERTENSION

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Abstract. A small series of hypertensive patients are accounted for. They are treated with propranolol orally twice daily instead of the common administration of 3-4 doses a day. In the first series of 10 patients on 2 doses replaces the maintenance of blood pressure control was tested by repeated BP measurements. No significant changes of BP were recorded during 10-hour observation period after the morning dose. Sublingual application of nitroglycerine in order to test the degree of adrenergic β -receptor blockade did not show significant increase of the pulse rate at the end of the observation period. The second series consisted of 39 previously untreated patients with mild to moderate hypertension. Propranolol given twice daily gave adequate BP control ($<170/100$) in 32 patients, while 7 had to be given additional diuretics. In 6 of the latter the maximum propranolol dose did not exceed 400 mg daily. The side effects were few and their frequency did not seem to be influenced by the 2-dose regimen.

Propranolol has been used as an antihypertensive agent for several years and its usefulness in this respect is well documented (6, 7). The usual administration of propranolol has been four doses a day orally. This is in accordance with pharmacological studies, which have shown that the adrenergic β -receptor blocking effect is maximal 1-3 hours after oral administration (8). It is also known that the blood levels of propranolol reach a peak 1-3 hours after oral administration, although there are wide individual variations in this respect (1, 6).

Pharmacodynamic studies of the metabolism of propranolol in man have shown that, after giving the drug orally at least one of the metabolites (4-hydroxy propranolol) has a β -blocking effect of about the same degree as propranolol and that other still unidentified metabolites are formed as well (5).

It is a well known fact that patients generally prefer to take their drugs only once or twice

daily and investigations have shown that with increasing number of tablets per day there is a greater risk that patients either forget or neglect to take every dose (4). It would therefore be desirable to reduce the daily number of doses of any drug, especially in long-term treatment, as for hypertension, provided that this is compatible with adequate control of BP.

As we already have positive experience of treating a large number of hypertensive patients with propranolol three times a day (3), it seemed natural to proceed with the present study in which propranolol was administered twice daily to 39 hypertensive patients and the sustained antihypertensive and β -blocking effects were tested in another 10 hypertensive patients.

MATERIAL AND METHODS

Ten patients with essential hypertension, all treated with propranolol given orally twice daily for at least two months, were included in the first series. All patients had benign hypertension, 7 with pronounced changes of grade II and five with grade I (Kotch-Wagener and Barker classification). Five patients were in stage I and five in stage 2 according to the classification of hypertension by WHO. The known average duration of the hypertension was 6.4 years (range 3 to 14 y). There were nine men and one woman, their average age being 50 years (range 61-28).

The daily dosage necessary for BP control had been reached by means of stepwise increments of dosage. The average daily dosage of propranolol was 256 mg (200-160 mg). The patients took the morning dose of propranolol at 6 a.m. The time schedule for BP measurement, pulse rate measurement and nitroglycerine tests is shown in Fig. 1. The study was made on an out-patient basis with the patients at their usual work between the tests. BP was recorded both in the supine position after 5 min rest and in the erect position after standing for 1 min. The pulse rate was measured in the standing position.

Tests for the degree of adrenergic β -receptor

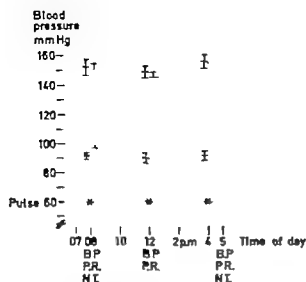


Fig. 1 The supine and standing BP and pulse rate (P.R.) at three different determinations. The nitroglycerine test (N.T.) was performed at 8 a.m. and between 4 and 5 p.m.

using the method described by Fitzgerald (2) were performed twice, 1 and 10 hours, respectively after the patients had taken their morning dose of propranolol. During the tests the patients were standing and pulse rates were counted during 6 min after sublingual application of 0.50 mg nitroglycerine. The highest pulse rate during the 6 min was then compared to the recumbent value immediately before the test.

Statistical comparison was made by the Student's *t*-test for the means of the differences between paired observations both when evaluating the changes of BP during the day and the changes of pulse rate in the tests for β -blockade.

The second series consisted of 39 earlier untreated hypertensive patients. The distribution according to WHO

stage, sex and racial changes is shown in Table 1. A routine diagnostic work-up including chest X-ray ECG and laboratory tests for proteinuria, serum electrolytes and serum creatinine was performed in all cases. Propranolol was instituted in a dosage of 40 mg twice daily for 3-5 days, whereafter the dose was doubled to 80 mg twice daily if normal BP was not obtained at the next return visit after another 10 days, the dose was again doubled, thus now reaching 320 mg as total daily dosage. To achieve satisfactory BP control, the propranolol dose was then increased stepwise (to 960 mg daily in one patient). In most cases, however the stepwise dose increments stopped at a lower level and hydralazine was usually instituted when BP control could not be achieved with 480 mg daily. Side-effects were only noted as spontaneous communications from the patients and are not asked for in standardized manner.

RESULTS

The average systolic and diastolic BP \pm S.E.M. and the pulse rate at 8 a.m., noon and 4 p.m. are given in Fig. 1. As will be seen, there were only minor fluctuations of the BP during the day. A statistical comparison by means of the Student's *t*-test showed that the BP changes were not significant at the 95% level. The changes of pulse frequency during the day were also statistically insignificant.

The nitroglycerine test for adrenergic β -receptor blockade failed to show any reduction of the degree of β -blockade. In the first test performed 1 hour after the morning dose there was an average increase of the pulse rate from 63 to 74 with no patient showing an increase of more than 14 beats/min. In the second test for β -receptor

Table 1 The patients subgrouped according to the WHO criteria and averaged changes BP control, average propranolol doses and side-effects are given

Figures within parentheses apply to the maximum dose used in poorly controlled patients

No. of pts.	Sex		FH			BP control ^a		Propranolol (mg/day)	Side-effects
	♂		0	I-II	III	Good	Poor		
<i>WHO stage I</i>									
23	18	4	14			13	1	250 (400)	
				9		8	1	(400)	1 headache (passing)
									1 muscular cramps
<i>WHO stage II</i>									
16	14	2	2			2		297	
				13		9	4	(320, 400, 320, 320)	2 dizziness
					1	0	1	(960)	

Below 170 systolic and 100 diastolic are regarded as good BP control.

blockade 10 hours after the morning dose the average pulse rate increased from 67 to 77 with no patient showing an increase of more than 18 beats/min. The pulse increase differences between morning and afternoon were not statistically significant at the 95% level.

The average systolic and diastolic BPs before and during propranolol treatment in the second series were 183/111 and 152/94 respectively. The differences were statistically significant. The therapeutic results in relation to severity of the hypertension are shown in Table I. A sustained reduction of BP below 170 systolic and 100 diastolic was judged as an acceptable control. With these criteria 32 patients (82%) were adequately controlled by propranolol in a 2-dose treatment programme. Seven patients (18%) could not be controlled by this treatment schedule. In two of these seven cases the final dose of propranolol was divided into a 4-dose schedule without achieving a further reduction of BP. These seven patients achieved normotension by addition of hydralazine. The distribution of the non-responders according to the severity of the hypertension, as well as the side-effects, are also shown in Table I.

None of the patients experienced any adverse effects of the increase of individual dose necessitated by reduction of the number of doses given.

DISCUSSION

There seems to be little need to discuss the convenience of giving only two doses instead of three or four a day. The risk that doses are forgotten or neglected is reduced and patients generally find it more convenient.

It may also be concluded that administering propranolol only twice daily does not seem to cause any undue fluctuations of the BP during the day as there was safe control throughout the day in all ten patients. There also seemed to be a sustained β -receptor blockade during the day as judged by the results of the nitroglycerin tests.

The present study also shows that it is possible to start antihypertensive treatment, using propranolol in a 2-dose regimen, with good results. These results suggest that a large propor-

tion of the hypertensive population seems to be well suited for propranolol given twice daily. When BP control cannot be achieved by propranolol given twice daily our policy has been to add hydralazine. As this drug needs to be given four times daily we have also changed the daily number of propranolol doses to four in these cases.

The prolonged antihypertensive effect of propranolol cannot be explained by the present study. One can only speculate about the possibilities that metabolites with longer plasma half-life than propranolol are responsible for the prolonged effect or that there is a saturation of the tissues with propranolol or its active metabolites to maintain the effect after the blood concentrations have been reduced.

Since the results of the present study seem to indicate that propranolol given only twice daily is compatible with safe control of BP and as the patients in the study clearly preferred this form of administration it is our intention to continue this principle.

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HAEMODYNAMIC EFFECTS OF β RECEPTOR BLOCKING AGENTS AND DIGITALIS IN ISCHAEMIC CORONARY HEART DISEASE WITH ANGINA PECTORIS

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Abstract. The haemodynamic effects of β -receptor blocking agents (propranolol and pindolol) alone and in combination with digitalis have been studied at rest and during exercise in 20 patients with coronary heart disease and angina pectoris. Right heart catheterization was performed and cardiac output was determined by the indicator-dilution technique. β -blockade induced at rest significant fall in heart rate, all other variables studied being fairly unchanged. During exercise the cardiac output and stroke volume were lower than before β -blockade, while the BP in the right ventricle, pulmonary artery and pulmonary artery wedged position rose. Propranolol gave slightly greater increase in pulmonary artery wedged pressures compared to pindolol, but no significant differences concerning the other variables studied. After administration of digitalis together with β -blocking agent the systolic and end-diastolic pressures of the right ventricle as well as the pulmonary artery wedged pressure during exercise were significantly lower than after β -blockade alone. Cardiac output, stroke volume and calculated left ventricular work index were all significantly higher while the product of heart rate and systolic BP was unchanged. These findings indicate that digitalis stimulates the negative inotropic effect of β -blocking agent but leaves the negative chronotropic effect unchanged. It is suggested that, when β -blocking agent is used in the treatment of angina pectoris, digitalis seems to be indicated when cardiac failure is likely to appear.

Numerous long-term studies have indicated that in cases of angina pectoris β -adrenergic blocking agents have a significant therapeutic effect as judged by a decrease in anginal attacks and decreased consumption of nitroglycerine (7). Acute experiments, moreover have shown that β -blockers improve exercise tolerance in such cases (2, 15, 24, 27). But there is now also substantial evidence that β -blocking agents can have adverse effects, most of them directly related to the pharmacological action of the substances. Haemodynamic studies have clearly shown that,

even in normal man, cardiac output and cardiac work are reduced at rest and during work by β -receptor blockade (6, 12, 13, 23). This reduction of cardiac work, caused by the negative inotropic and chronotropic properties of the β blockers, seems to be the mechanism for their therapeutic effect in cases of anginal pain. But as a consequence, in patients with valvular lesions and also in cases with impaired myocardial function near the borderline of failure treatment with β -blocking agents may give rise to symptoms of congestive cardiac failure (5, 26). However the pharmacological properties of the various β -blockers seem to differ somewhat in this respect. Alprenolol (1) and pindolol (4)—in contrast to propranolol—are reported to have an "intrinsic activity" that is a weak stimulating action on heart rate and contractile force. It has been claimed that drugs with "intrinsic activity" are less likely to precipitate heart failure. Thus Lichten and Mocetti (21) found a stronger cardiodepressive activity during exercise in man using propranolol compared to pindolol. So far however no further evidence has been presented to support a difference of clinical significance between these two substances.

To counteract the risk of myocardial failure it has been recommended that, in addition to a β -blocker digitalis should be given in cases in which signs of congestive cardiac failure appear or may be expected to appear. As yet, however the central haemodynamics during combined administration of a β -blocker and digitalis have not been studied fully and it has been discussed whether to some extent, the beneficial effect of a β -blocker on the oxygen

Table 1. Median effects of β -adrenergic blockade on central haemodynamics at rest and during exercise

C = control study performed before administration of the β -blocking agent (phase I), Δ = changes in median values in connection with administration of the drug (phase III) compared with phase I, p = levels of significance, ns = not significant

	Rest				p C- β -block.	p Propranolol- pindolol
	Propranolol	Pindolol	C	Δ		
Heart rate (beats/min)	72	-8	78	-6	<0.01	ns
O ₂ pm consumption (ml/min)	257	6	267	-2	ns	ns
Cardiac output (l/min)	5.2	-0.5	6.0	-0.4	ns	ns
Stroke volume (ml)	67	3	78	2	ns	ns
A-V oxygen difference (ml/l)	48.2	3.3	43.3	4.9	<0.05	ns
BP (mmHg)						
Right ventricle, systolic	27	-1	23	-2	ns	ns
End-diastolic	5	0	5	0	ns	ns
Pulmonary artery, systolic	22	1	20	0	ns	ns
Diastolic	9	0	9	0	ns	ns
Mean	13	0	16	-2	ns	ns
Wedge	10	-1	8	0	ns	ns
Brachial artery, systolic	142	-2	148	1	ns	ns
Diastolic	79	1	89	1	ns	ns
Mean	112	0	117	-2	ns	ns
Pressure time/min (mmHg/sec/beat)	2 584	-356	3 072	-269	<0.01	ns
Systolic ejection rate (ml/sec/cm ²)	130	7	135	-3	ns	ns
Left ventricular work index (kgm/min/m ²)	4.6	-0.4	5.6	-0.3	ns	ns
Pulmonary vascular resistance index (dyn/sec/cm ⁵)	2 199	-172	2 056	-394	ns	ns
Systemic vascular resistance index (dyn/sec/cm ⁵)	37	3	35	3	ns	ns

heart in cases of anginal pain is reduced after digitalization. The present study was made on cases of coronary heart disease with angina pectoris and consists of two parts. In the first the effect of propranolol on the central haemodynamics is compared to that of pindolol. In the second the study is extended to deal with the effect of digitalis in addition to β -blockade.

MATERIAL

Twenty subjects, 11 men and 9 women, aged 47-63 years, with ischaemic coronary heart disease, participated in the study. All fulfilled the requirement that, in work on a bicycle ergometer (1) given, load, ideally evaluated load, they had typical angina pectoris and an abnormal ECG reaction with ST depression.

METHODS

Right heart catheterization was performed from the left arm using Cournand double catheter. A teflon catheter was introduced percutaneously into the right brachial artery. BP and cardiac output were measured at rest and during supine exercise. BPs were recorded with pressure transducers (Elema-Schönander, Sweden), using the mid-thoracic flow as the reference level. Cardiac output was determined with the indicator-dilution technique using

indocyanine green (Cardiogreen) as indicator. The dye was injected into the pulmonary artery and the dye concentration was analysed in blood taken from the brachial artery and passing a Waters cassette densitometer (CX300). Heart rate was obtained from ECGs. Exercise was performed in the supine position on an electrically braked bicycle ergometer (Elema-Schönander).

The oxygen content of blood was calculated from duplicate spectrophotometrical determinations of oxygen saturation (16), and the Hb concentration was calculated by the cyanmethaemoglobin method (17).

Calculations

Left ventricular work index (kgm/min/m²):

$$\left[\frac{\text{brach. art. syst. mean - pulm. art. wedge pressure}}{\text{cardiac index}} \right] \times 1.36 / 1000$$

Pressure time/min (mmHg/sec/min):

$$[\text{brach. art. syst. mean} - \text{syst. ejection period}] \times \text{heart rate}$$

Pulmonary vascular resistance index (dyn/sec/cm⁵):

$$\frac{\text{pulm. art. mean - pulm. art. wedge pressure}}{\text{cardiac index}} \times 1.332$$

Systemic vascular resistance index (dyn/sec/cm⁵):

$$\frac{\text{brach. art. mean}}{\text{cardiac index}} \times 1.332$$

Statistical method

Non-parametric analyses of differences (this sub-*cm* (after-before treatment)) has been performed. A *crude* values are given as medians.

RESULTS

Effect of β -adrenergic blockade (Table I)

Twenty patients were studied according to phases I-III, 10 were given propranolol and 10 pindolol.

Clinical observations. Of the 20 subjects, all of whom accordingly had angina pectoris in the preparatory work tests, 18 had symptoms of this type when they exercised at the same load during phase I. The two subjects, one in the pindolol group and the other in the propranolol group, who did not have symptoms during the first phase did not have them either when exercise was repeated after administration of the β -blocking substance. Of the other 18 only 9 in the pindolol group and 3 of 9 in the propranolol group had angina pectoris during exercise after administration of the β -blocking substance (phase III). The difference in this respect between the two compounds is not significant. In four cases symptoms of congestive cardiac failure in the form of dyspnoea and suspicious rales appeared at the end of the exercise during β -blockade. Two of these cases had the highest pulmonary arterial wedged pressures (32 and 31 mmHg, respectively) recorded in the whole material.

Haemodynamic results. At rest the heart rate

Confidence intervals for median drug effects have been computed according to Nahr (22).

Procedure

The patients were examined in the recumbent position. The procedure was divided into five phases.

I. Recording of BP cardiac output and arterial-pulmonary artery (a-v) oxygen differences at rest and during work. The work lasted for about 15 min. The work load had been estimated previously and was set at a level such that the patient could exercise for at least 12 min before he started to feel mild angina pectoris. The load required to induce pain while working in the recumbent position varied for the 20 patients between 75 and 300 kpm/min.

II. The β -adrenergic blocking agent was given I. in doses of 0.07 mg propranolol (ICI) or 0.007 mg pindolol (Sandoz)/kg b.wt. in random order. The patient then rested for 30 min.

III. The procedure presented in phase I was repeated.

In the second part of the investigation the effect of digitalis was studied after β -blockade in 10 of the cases. Ph. had received propranolol and 5 pindolol.

IV. Digitalis, 1.2 mg desacetil kanatoxol C (Sandoz), was given intravenously immediately after phase III was finished. After 30 min half of the dose of the β -blocking agent given in phase II was added. By this time 70-80 min had elapsed since the first dose was administered and the aim was to make the degree of β -blockade in phases III and V as similar as possible.

V. In this phase, which started 30 min after the administration of digitalis, the schedule was the same as in phases I and III.

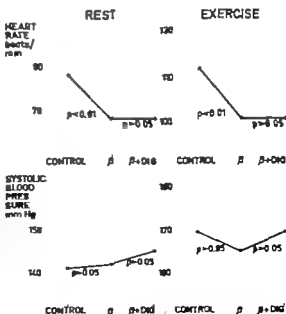


Fig. 1 Effect of β -adrenergic blockade alone and combined with digitalis on heart rate and brachial artery systolic BP at rest and during exercise. Median values.

Table II Median effects of β -adrenergic blocker alone and in combination with digitalis on central haemodynamics during exercise

M_L and M_U —lower and upper bounds for the median effect. Other symbols as in Table I. Confidence level 95 %. The values for β -blockade represent combined results with propranolol and pindolol

	Control (phase I) median value	β -blockade			Phase I-III (p)	β -blockade + digitalis			Phase III-V (p)	Phase I-V (p)
		Δ	M_L	M_U		Δ	M_L	M_U		
Heart rate (beats/min)	112	-13	-23	-8	<0.01	-12	-23	-9	ns	<0.01
Oxygen consumption (ml/min)	820	-34	189	38	ns	-1	-73	64	ns	ns
Cardiac output (l/min)	9.8	-3.7	-3.3	-0.7	<0.01	-0.3	-1.1	-0.4	<0.01	ns
Stroke volume (ml)	89	-7	-31	7	ns	3	1	18	<0.01	<0.01
A-V oxygen difference (ml/l)	87.7	10.9	6.1	24.0	<0.01	3.0	-0.3	9.7	<0.01	<0.01
BP (mmHg)										
Right atrial, systolic	42	7	-1	12	ns	3	-1	3	<0.05	ns
End-diastolic	7	2	0	6	ns	0	-2	1	<0.01	ns
Pulmonary artery, systolic	39	8	3	11	<0.05	8	-3	2	<0.05	ns
Diastolic	15	6	2	8	<0.01	6	-3	2	<0.01	ns
Mean	22	7	3	11	<0.01	1	-1	2	<0.01	ns
Brachial artery, systolic	169	-4	-28	4	ns	-3	-13	3	ns	ns
Diastolic	85	1	-5	4	ns	2	-5	3	ns	ns
Mean	112	-2	-5	4	ns	-1	-8	8	ns	ns
Pressure time/min (mmHg/sec/beat)	4299	-279	-1042	-80	<0.01	-725	-1165	-304	<0.05	<0.01
Systolic ejection rate (ml/sec/cm ²)	175	-13	-60	13	ns	27	6	38	<0.01	<0.01
Left ventricular work index (kg/m/m ²)	9.4	-2.4	-3.4	-1.2	<0.01	-0.3	-1.2	0.7	<0.01	ns
Pulmonary vascular resistance index (dy \cdot sec/cm ⁻⁵)	1.837	813	-1172	1192	ns	239	-942	1473	ns	ns
Systemic vascular resistance index (dy \cdot sec/cm ⁻⁵)	21	5	1	9	<0.05	2	-1	3	ns	ns

decreased significantly in connection with β -adrenergic blockade. No effect was found on BP or cardiac output, but a small increase in A-V oxygen difference. Calculated pressure time per min decreased slightly. No significant difference was found in any variable between propranolol and pindolol.

During exercise the increase in heart rate was significantly less pronounced after β -adrenergic blockade than in the control study. Cardiac output and stroke volume were lower and a-v oxygen difference higher after β -adrenergic blockade. BPs of the right atrium and mean pulmonary artery as well as in the pulmonary wedged position rose to significantly higher values, while the systemic arterial BP was unchanged. Calculated left ventricular work index and pressure time per min were significantly lower. Compared to pindolol, propranolol gave a slightly greater increase of the pulmonary artery wedged pressure. No other significant difference was found between the two β -adrenergic blocking agents.

Combined effect of digitalis and β -adrenergic blockade (Table II)

As mentioned earlier, phases IV and V were studied in 10 of the patients included in phases I-III. In this smaller material there were no significant differences between the effects of the two β -adrenergic blocking drugs. Therefore it was considered justified to look upon the cases as one group in the statistical analyses of the effect of digitalis.

Clinical observations. There was no difference in the appearance of angina pectoris after administration of digitalis. Three of the ten cases developed angina pectoris during the exercise test with β -blockade. They also had this symptom during exercise after digitalization. However, in the two cases in this group, in which clinical symptoms of cardiac failure appeared during exercise with β -blockade, no such symptoms appeared during exercise after the administrations of digitalis.

Haemodynamic results. At rest no significant

difference was found in any variable studied when digitals and β -blocking agents were combined as compared to β -blockade alone (Figs. 1-3).

During exercise the heart rate was identical to the findings after β -blockers alone (Fig. 1). The cardiac index rose to higher values after digitals and β -blocker than when exercise was performed after β -blockade alone (Fig. 2). In fact, administration of digitals during β -blockade increased cardiac output during exercise to a level which did not differ significantly from that of the control study. Stroke volume during exercise was significantly higher after combined administration of digitals and β -blocker than in either the control study or when a β -blocker had been given alone. A V oxygen difference increased compared to the level during β -blockade alone and reached much the same values as in the control study.

Right ventricular systolic and end-diastolic pressures, pulmonary artery pressures (systolic, mean, diastolic) and mean pulmonary artery wedged pressure were all significantly lower during exercise performed after combined administration of digitals and β -blocker than after β -blockade alone (Fig. 3). In fact, the intracardiac pressures measured after digitals and β -blocker were the same as in the control study.

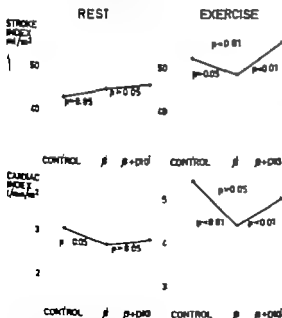


Fig. 2 Effect of β -adrenergic blockade alone and combined with digitals on stroke and cardiac index at rest and during exercise. Median values.

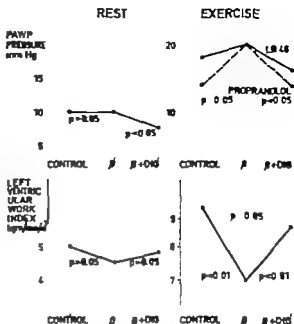


Fig. 3 Effect of β -adrenergic blockade alone and combined with digitals on pulmonary arterial wedged pressure and calculated left ventricular work index at rest and during exercise. Median values.

Calculated left ventricular work index and mean systolic ejection rate were significantly higher during exercise after the combined administration of digitals and β -blocking agent than after β -blockade alone. Pressure time per minute was also significantly lower.

DISCUSSION

The present effects of β -adrenergic blocking agents on the haemodynamic response to exercise conform with the findings of others (10, 19, 21). In other studies the left ventricular first derivative (10) and the velocity of myocardial contraction (25) were found to decrease. The data obtained from these studies thus convincingly indicate that β -adrenergic blockade alters several of the major determinants of myocardial oxygen requirements, as already suggested by Epstein and Braunwald (11). This suggestion was recently confirmed by direct measurements of the oxygen content in the coronary sinus in the dog (2). The reduction of myocardial oxygen consumption thus obtained probably explains the beneficial effects of β -adrenergic blockade in angina pectoris.

not attend the survey because of medical treatment at the time were studied separately.

If a person had been sick-listed on some occasion (number of occasions not considered) before the invitation to the survey this was recorded. Among the diagnoses we recorded only urinary calculi, "pyelitis" — pyelonephritis, urinary tract infection, and "others". The latter group included, for instance, proteinuria of pregnancy and glomerulonephritis. The diagnoses are listed here in order of priority.

The diagnosis of hypertension occurred in only a few cases. The explanation would be that the doctors usually treat this condition with drugs without reporting the patient sick. Complications of hypertension, such as cardiac decompensation, are in most cases probably recorded under the above listed diagnoses without statement of hypertension. Therefore, hypertension was not included among our diagnoses.

Table 1 shows the incidence of each diagnosis as percentage of the total number of persons in the respective diagnostic group and the percentage distribution by sex. The average total figure for those screened was 9.4% that is, 19.0% higher than for those who did not attend the screening. The difference is due to a higher sickness rate for the screened than for the non-screened women in the groups of "pyelitis" — pyelonephritis, urinary tract infection, and cystitis. The figure for those who did not take part in the survey because they were under medical treatment was higher than for the other groups, namely 13.5%. As expected, the incidence of diseases other than urinary calculi was higher in women than in men.

The fact that the percentage for sick-listing on the said diagnoses was higher for the screened than for the non-screened persons could imply that those who had experience of previous illness tended to be more interested in health surveys.

Examination for other pathological conditions

For psychological reasons it seemed necessary to extend the medical examination and include other pathological conditions, such as gastrointestinal disorders.

ACKNOWLEDGEMENTS

The treatment of the material was made possible by grants from Malmöhus Local Social Insurance Office, and the publication of the result by grants from AB Gambrø, Lund, and Mr John-Henry Sager Stockholm.

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A POPULATION STUDY ON RENAL AND URINARY TRACT DISEASES

II. Urinary Deposits Bacteriuria and ESR on Screening and Medical Examination of Selected Cases

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Abstract. Urine specimens have been examined for urinary deposits by the conventional clinical method, that is, by centrifugation and counting of formed elements per high power field (HPF). The criteria for pyuria were at least 5/11 and 21 WBC/HPF. Conventional urine specimens, without instructions as to midstream collection, showed pyuria by the said criteria in an average of 8.4%, 4.2% and 1.3% respectively of the men and 40.3%, 21.0% and 8.8% of the women. The screening comprised 1235 persons, 47.7% being men and 52.3% women. The clean-voided midstream technique was applied in the screening of 2643 persons, 50.1% men and 49.9% women. Pyuria was noted in 3.2%, 1.6% and 0.5% respectively of the men and in 17.2%, 10.0% and 3.7% of the women. Thus, the midstream technique reduced the incidence of pyuria according to all three criteria by about 60% both in men and in women. In the literature this technique is generally stated to be necessary only in women in order to avoid admixture of formed elements from the urethra and the external genitalia. Screening and subsequent medical examination of clean-voided midstream specimens revealed pyuria in altogether one-third of the cases on both occasions, at screening alone, or at medical examination alone. White cell casts and clumps of leucocytes occurred in only a few cases. By the criteria of at least 3/7 and 11 RBC/HPF the prevalence of haematuria in the first mentioned series was 4.8%, 1.5% and 0.2% respectively for the men and 10.2%, 3.0% and 1.3% for the women; in the latter series (midstream specimens) the comparable figures were 3.9%, 1.0% and 0.6% for the men and 5.7%, 1.3% and 1.4% for the women. While the midstream technique reduced the prevalence of haematuria by about 45% for the women, using the criteria of 3 RBC/HPF the other figures agreed fairly well for the two series; reservations are made for relatively small number of observations in some instances. Labix gave in too many cases false negative results for haematuria. Screening and/or subsequent medical examination revealed haematuria in only about one-sixth of the cases on both occasions, in about one-third at medical examination alone, and in the rest on screening alone. Proteinuria was also present in 10-15% of the specimens with pyuria. Significant bacteriuria was recorded

in 13% of the specimens with slight pyuria, in 30% of those with marked pyuria, and in 4% of those without pyuria. There seemed to be no correlation between the presence of pyuria and that of significant bacteriuria. *L-forms* were demonstrated in only 0.5% and various types of *Mycoplasma* in 30% of the specimens. Tubercle bacilli were found in two cases of pyuria. Every three persons with and every five without pyuria had elevated ESR. Thus, the correlation pyuria-elevated ESR was of limited diagnostic value.

Pyuria and microscopic haematuria are considered to be of great importance as symptoms of renal and urinary tract diseases. According to most textbooks, the minimum diagnostic criterion is at least 5 white and at least 3 red cells per HPF. Probably because slight pyuria is also frequently present in persons who have no other signs of the said diseases, some authors allow up to, for instance, 10 WBC/HPF without establishing the diagnosis of pyuria. Pyuria is said to occur mainly in bacteriuria. Hence, screening for pyelonephritis and urinary tract infection is often limited to urine cultures, dip-slides, or chemical tests. Clean-voided midstream specimens are recommended, in particular to avoid admixture of white cells and other material from the lower urinary tract and external genitalia and bacterial contamination of cultures and dip-slides, but the technique is not always used in practice. It is mentioned in the literature that the presence and the degree of pyuria can vary from one examination to another in the same individual. This can also be the case in bacteriuria. Yet, at routine medical examination single tests for bacteriuria and pyuria often form the basis for diagnosis and therapy.

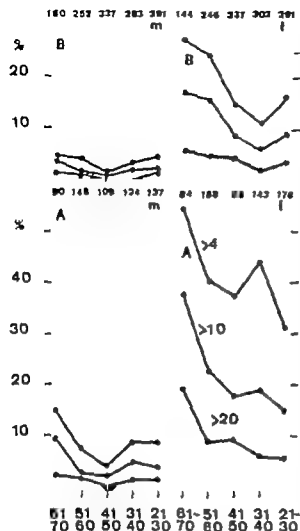


Fig. 1. Prevalence on screening of pyuria by the criteria of at least 9, 11 and 21 WBC/HPF respectively (A) conventional urinary specimens, (B) clean-voided midstream specimens. Distribution by sex and age.

We report here the findings of urinary deposits and bacteriuria on screening of 3998 persons and at medical examination of 276 of them. The results illustrate everyday technical problems, such as the importance of clean-voided midstream specimens, pyuria and bacteriuria, and elevated ESR. The latter constellation is believed to be of great diagnostic significance in chronic pyelonephritis.

In a subsequent paper (3) we will report the results of a comparison between the occurrence of bacteriuria and pyuria in urinary specimens obtained by percutaneous suprapubic aspiration

and by collection of clean-voided midstream specimens immediately after the puncture.

The reader will find that the total number of observations varies between the reported series. The reason is that the figures have been taken from different combinations at computer analyses of various questions. This fact does not influence the results.

METHODS

Urine cultures were performed at the Department of Microbiology of the University Hospital and other laboratory procedures at the laboratory of the Health Service dispensary.

In previous papers we have reported the technique of selection of persons for screening and medical examination (2) and the screening technique (1). The screened persons mailed to our laboratory tubes with urine from the first micturition in the morning after restricted fluid intake. To the tubes had been added two drops 10% thiomersal-sodium solution to inhibit bacterial growth. The transport by post, at current temperatures took one to several days. Women were instructed not to collect urine specimens during the menstrual period.

Instructions as regards clean-voided midstream specimens were not applied in the first screening period over the first six months of 1969. In this period, 3... of the whole series of 3998 persons were screened. Such instructions were given during the rest of the screening period from Aug. 1969 to March 1971.

During the latter screening period the following methods were used: (1) the medical examination of persons with pathological findings on screening and/or questionnaire data on symptoms and diseases. Clean-voided midstream specimens were collected six hours after the previous micturition preceded by restriction of fluid intake (1). These instructions were not applied in the first part of the series. The urine specimens were centrifuged for 5 min at 3000 rpm. The sediment was examined under the microscope, magnification 320. The pH of the urine was determined with Labster (Ames). The strip is easy read and seems to be reliable within the pH range of interest in this study.

A previous paper (2) gives a report of the selection of persons for screening and medical examination and their distribution by sex and age.

Screening of 1255 consecutive conventional urine specimens without instructions as to clean-voided midstream specimens

The series comprised 598 men (47.7%) and 657 women (52.3%). Figs. 1 and 2, lower halves (A), show the distribution by sex and age of those who had pyuria and haematuria. Eighty men and 84 women were over 60 years, in the age groups 51-60 and 21-30 the figures were 109-137 and 96-176 respectively.

Fig. 1 shows the prevalence of pyuria by the criteria of at least 5 11 and 21 WBC/HPF respectively. Both in men and in women the figures are highest in the highest age group (14.9% 9.9% 1.2% for the men and 54.7% 38.0% 19.0% for the women). For the women the curves fall, on the whole, gradually to a minimum in the lowest age group (31.2% 14.8% 5.7% respectively), while for the men the minimum is noted in the age group 41-50 (3.7% 1.8% 0%). By the said criteria the incidence of pyuria averaged 8.4% 4.2% and 1.3% respectively for the men and 40.3% 21.0% and 8.8% for the women.

Fig. 2 shows the prevalence of haematuria by the criteria of at least 3 7 and 11 RBC/HPF respectively. For the men the figures are 5.0% 2.5% and 0% in the highest age group, 2.4% 1.6% and 0% in the age group 31-40 and 3.7% 1.5% and 0% in the age group 21-30; for the women the figures are 4.8% 0% and 0% in the highest age group and 10.9% 2.9% and 1.2% in the lowest age group. By the same criteria, the incidence of haematuria averaged 4.2% 1.5% and 1.2% for the men and 10.2% 3.0% and 1.3% for the women.

Screening of 2 643 consecutive persons providing clean-voided midstream specimens

The series comprised 1 323 men (50.1%) and 1 320 women (49.9%). The upper halves of Figs. 1 and 2 (B) show the distribution by sex and age. In the highest age group there were 160 men and 144 women and in the others 252-337 men and 246-337 women.

Fig. 1 shows the incidence of pyuria by the criteria of at least 5 11 and 21 WBC/HPF respectively. For the men the figures are highest in the highest age group (4.3% 3.7% 1.2%) and in the lowest age group (4.4% 2.0% 1.0%) with a minimum in the age group 41-50. For the women the incidence is highest in the highest age group (27.1% 16.7% 5.6%), falls to a minimum in the age group 31-40 (11.3% 5.7% 1.7%) and rises again in the lowest age group (16.1% 8.9% 3.4%). By the said criteria, the incidence of pyuria averaged 3.2% 1.6% 0.5% for the men and 17.3% 10.0% 3.7% for the women.

The latter figures make up the following differences of the comparable averages in the first

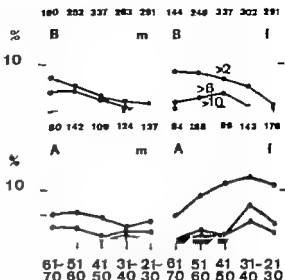


Fig. 2. Prevalence on screening of haematuria by the criteria of at least 3 7 and 11 red cells/HPF respectively (A) conventional urinary specimens, (B) clean-voided midstream specimens. Distribution by sex and age.

part of the series: 38.1% 38.1% and 38.5% for the men and 42.9% 13.1% and 41.1% for the women. Accordingly the midstream technique reduced the incidence of pyuria by about 60% irrespective of the criteria used.

Fig. 2 shows the prevalence of haematuria by the criteria of at least 3 7 and 11 RBC/HPF respectively. The figures for the men are highest in the highest age group (7.4% 4.3% 1.2% respectively) and fall gradually to a minimum in the lowest age group (2.3% 0.6% 0.3%). The curves for the women run a similar course, reaching a maximum in the highest age group (8.4% 2.1% 2.1%) and a minimum in the lowest age group (1.7% 1.0% 0.7%). By the said criteria, the incidence of haematuria averaged 3.9% 1.0% and 0.6% for the men and 5.7% 2.3% and 1.4% for the women. Many of the percent ages are of course uncertain, because of too small a number of persons in the group; nevertheless it looks as if the midstream technique would have reduced the incidence of haematuria relatively little, unlike the result for pyuria.

Coexisting pyuria and haematuria on screening and/or subsequent medical examination

This study comprised only persons from the latter part of the series. Hence clean-voided midstream

Table 1. Correlation between pH and occurrence of pyuria (at least 5 WBC/HPF) on screening and medical examination

Medical examination		Screening						
		pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11
pH 5	Pyuria	463	367	37	43	6	10	—
	—	144	81	37	14	7	1	4
pH 6	Pyuria	321	234	111	43	18	12	—
	—	67	29	23	9	3	2	1
pH 7	Pyuria	334	232	33	53	3	12	1
	—	53	20	15	12	4	1	1
pH 8	Pyuria	15	18	1	3	—	1	—
	—	4	3	1	—	—	—	—
Total		1401	976	169	177	33	39	7

specimens were examined both by screening and clinically.

On screening and/or medical examination 336 persons had at least 5 WBC/HPF in the sediment, the interval between the examinations was 1–14 days and 15 days or more respectively (40.5% and 59.5%). A total of 221 persons (65.8%) had pyuria on screening and 99 (44.8%) of them at the subsequent examination as well, in the rest, 55.2% it was no longer present. When the interval between screening and medical examination was at most 14 days, pyuria persisted in 50 out of 93 persons, or in 53.8% and with a longer interval it persisted in 38.3%.

In 115 (34.2%) of the 336 persons pyuria was not present until the medical examination, it was noted within 14 days of screening in 37.4% and after a longer interval in 62.6%. The percentage relation between the results of the two examinations did not change when the wider cri-

teria of at least 11 and 21 WBC/HPF were used.

On screening and/or subsequent examination 180 persons revealed haematuria, at least 3 RBC/HPF with intervals of 1–14 days in 38.3% and with longer intervals in 61.7%. In all 101 (56.1% of the total number) had haematuria on screening and in 35 (34.7%) it was present at the subsequent examination as well, in the rest it was no longer present. With an interval between screening and examination of at most 14 days, haematuria persisted in 20 or 19.8% and with longer intervals in 14.9%.

In 79 of the 180 persons (43.9%) haematuria was not present until the medical examination, in 29.1% of these 79 it was revealed at examination within 14 days of the negative screening and in the rest after longer intervals. The percentage relation between the results of the two examinations was mainly unchanged whether the criterion was at least 3.7 or 11 RBC/HPF.

Table I shows the distribution by pH of specimens from clinically examined and screened persons and the presence of pyuria, at least 5 WBC/HPF in the different groups. The two series differed in that the specimens for screening were mailed to our laboratory in tubes containing a preservative while those for clinical examination were examined directly after micturition.

The percentage distribution by the pH of the specimens was as follows, the first figure referring to the screened series and the second figure in parentheses to the clinically examined series: pH 5 81.4 (60.7) % pH 6 15.0 (27.7) % pH 7 3.3 (27.6) % and pH 8 0 (1.4) %.

The prevalence of pyuria was as follows. pH 5 14.8 (23.7) % pH 6 15.7 (17.3) % pH 7 15.2 (13.7) %. There were no specimens with pH 8 at the screening. The pH values of all the specimens are lower than the values that are critical with respect to the tenacity of the white cells.

At pH 5 pyuria was absent both on screening and on the subsequent examination in 70.3% (and at pH 6 in 66.2%). Pyuria was present on screening alone in 7.1% (15.4%), at the clinical examination alone in 14.6% (13.8%), and both at screening and at clinical examination in 7.1% (4.6%).

Because of the small number of observations, some percentages are uncertain. For this reason, the result at pH 7 is not commented upon.

It seems thus probable that the postal transport

Table II. Coexisting pyuria and haematuria at medical examination

RBC/HPF	WBC/HPF				
	0–4	5–10	11–20	21	Total
0–2	890	204	64	84	1262
3–6	71	22	9	16	118
7–10	25	4	4	4	37
11–15	11	5	1	3	20
20–	30	9	1	6	46
Total	1027	244	99	113	1483

of the screening specimens did not reduce the incidence of pyuria to any substantial degree. This is borne out by observations *in vitro*.

Most specimens were concentrated. The presence of red cell fragments was considered at the microscopical examination.

Coexisting pyuria and haematuria at clinical examination

Table II shows the coexistence of varying degrees of pyuria and haematuria in 1483 clean-voided midstream specimens collected at clinical examination of persons included in the latter part of the screened series. 60.0% of the specimens showed no pathological sediment.

By the criteria of at least 5/11 and 21 WBC/HPF respectively pyuria was present in 25.1%, 11.3% and 4.5%. By the criteria of at least 3/7 and 11 RBC/HPF haematuria was present in 9.2%, 4.5% and 2.8%.

At least 5 white and at least 3 red cells/HPF were noted in the same specimen in 5.7% of the total number (14.1% of those with pyuria) at least 11 white and at least 7 red cells occurred in 1.3% (3.2%).

Presence of white cell and red cell casts in pyuria and in haematuria on screening and on examination

Casts made up of white cells were noted in 2.0% of the specimens showing pyuria (at least 5 WBC/HPF); the figure at clinical examination was 5.6%. The comparable figures for red cell casts in haematuria (at least 3 RBC/HPF) were 2.7% and 1.4%.

The lower frequency of white cell casts on screening, as compared with that noted at medical examination, might indicate that the casts, to some extent, had been destroyed during the postal transport. The assessment should be made with reservation for the small number of observations.

Occurrence of bacteriuria and pyuria at medical examination

Table III shows a series of 937 specimens, 22.9% of which revealed pyuria (at least 5 WBC/HPF). The first culture yielded significant bacteriuria, that is, more than 10^5 bacteria/ml, in 7.8% and two cultures gave similar results in 4.9% of the specimens.

Table III *Prevalence of bacteriuria (first culture and two cultures respectively) at medical examination of specimens with and without pyuria*

No. of WBC	No. of specimens		Significant bacteriuria			
			First culture		Two cultures	
	No.	%	No.	%	No.	%
<5	722	77.1	28	3.9	14	1.9
>5	114	12.2	15	13.2	10	8.8
>11	30	5.3	14	23.0	8	16.0
>21	51	5.4	16	31.4	18	27.5
Total	937	100.0	73	7.8	46	4.9

For specimens with 5 to 10 WBC/HPF one culture was positive in 13.2% whereas for those with at least 21 WBC/HPF the figure was 31.4%. Repeated cultures were positive in 8.8% and 27.5% respectively.

Among 722 specimens without pyuria one culture was positive in 3.9% and repeated cultures were positive in 1.9%.

Table IV refers to a series of 1116 specimens. Pyuria was present in 18.8%. Dip-slides (Inculator® MacConkey's medium) revealed significant bacteriuria in 18.0% of the specimens with pyuria and in 2.2% of the rest. The incidence of bacteriuria rose with increasing degree of pyuria, at least 5 and at least 21 WBC/HPF from 9.3% to 34.0%.

Bacterial growth of 10^2 – 10^4 bacteria per ml, that is, subsignificant bacteriuria, was noted in 6.4% of the specimens without pyuria and in 8.4% of those with pyuria. The degree of pyuria was of no consequence here.

Table IV *Prevalence of no growth, subsignificant bacteriuria and significant bacteriuria (dip-slide MacConkey medium) at medical examination of specimens with and without pyuria*

No. of WBC	Bacterial count					
	No growth		10^2 – 10^4 /ml		$>10^5$ – 10^8 /ml	
	No.	%	No.	%	No.	%
<5	828	90.3	58	6.4	20	2.2
>5	103	30.3	111	10.2	11	9.3
>11	31	68.9	3	6.7	11	24.4
>21	28	39.6	3	6.4	16	34.0
Total	982	88.0	76	6.8	58	5.2

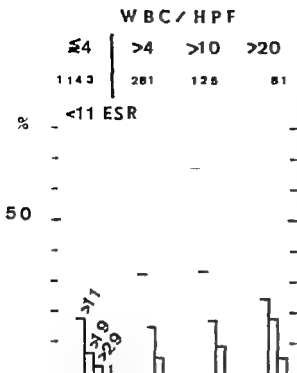


Fig. 3 Correlation between pyuria by the criteria of at least 5 WBC/HPF and 1 WBC/HPF respectively and ESR by the criteria: at most 11 mm/h (normal), >11–19 and >19 respectively

Presence of L-forms and *Mycoplasma* at medical examination of specimens with pyuria

A series of 193 specimens from 150 persons with pyuria were examined for L-forms, 0% were (23.8% of the specimens) and 78.0% women (76.2%). The majority were abacteriuric. None was undergoing treatment for urinary tract infection or pyelonephritis. The age groups were as follows: 61 and over 41–60 41–50 31–40 and 21–30. The number of women in each group was 41 35 19 19 and 35 respectively and of men 9 2 2, 8 and 12. L-forms were demonstrated in only one specimen from a woman with diagnosed cystitis.

For *Mycoplasma hominis* and other types 254 specimens from 175 persons with pyuria were examined, 22.9% were men (23.2% of all the specimens) and 77.1% women (76.8%). Of the afore mentioned 150 persons 148 were included in this series. The numbers in each of the five age groups were 33 7 21 20 and 113 women and 11 4 3 7 and 15 men 71 (30.0%) of the specimens were

positive. *Mycoplasma* type T was found in 53.5% of the positive specimens, type hominis in 19.7% both type T and hominis in 19.7% and other types in 7.1% of the positive specimens. Of the positive specimens 18.3% were obtained from men and 81.7% from women. Guinea pig tests were made on 175 specimens with sterile pyuria. Two cases of tuberculosis were revealed.

Correlation between pyuria and ESR at medical examination

Fig. 3 shows the prevalence of pyuria in a series of 144 persons, the criteria being at least 5 WBC/HPF (19.7% of those examined), at least 11 (9.8%) and at least 1 (4.3%). Clean-voided midstream specimens were examined. ESR was normal (at most 11 mm/h) in 79.5% of the series, at least 12 mm in 20.5% at least 20 mm in 8.3% and at least 30 mm in 2.5%.

Summarizing, the ESR was elevated (at least 12 mm/h) in 17.7% of 1143 persons without pyuria and in 3.6% of 281 with pyuria (at least 5 WBC/HPF) at higher degrees of pyuria the figures were 33.6% in 1.5 persons with at least 11 WBC/HPF and 4.6% in 61 with at least 1 WBC/HPF.

With reservation for the great difference in the number of persons between the groups, it can be generally concluded that approximately every fifth person without pyuria and every third with pyuria had an elevated ESR and that there was no correlation between the degree of pyuria and elevation of the ESR. These figures seem to be of practical interest, since pyuria and elevation of the ESR are usually considered to be criteria of active pyelonephritis. The question to what extent extrarenal pathological conditions can have caused elevation of the ESR will not be discussed.

Table V Correlation between the number of red cells/HPF and the results of Labstix as a reagent to haematuria

RBC/HPF	No. of specimens	Labstix ()				
<3	5683	99.5	0.4	0.1	0.05	
3–6	305	94.3	3.0	1.6	0	
7–10	85	87.1	9.4	2.4	1.2	
10–19	37	81.1	16.2	2.7	0	
20>	78	41.0	23.1	25.4	10.3	

here. This problem was considered at the subsequent clinical examination of individual cases, whether or not pyuria was present.

The constellation of pyuria and elevated ESR is, thus, of limited diagnostic value per se.

Correlation between pyuria and proteinuria in the same individual at screening and medical examination: specimens with haematuria excluded

Specimens from 1456 persons on screening and at medical examination were studied. On any of the two occasions 1004 (70.0%) of them had neither pyuria (at least 5 white cells) nor proteinuria (positive sulphosalicylic acid reaction and/or Heller's test). Specimens with haematuria (at least 3 RBC/HPF) were not included in this series.

Screening revealed pyuria in 15.2% of all the persons in the series and proteinuria in 1.7%. The comparable figures at clinical examination were 18.6% and 2.8%.

Proteinuria was present on both occasions in 0.9% on screening alone in 0.8% and on medical examination alone in 2.6%. Thus, the incidence of proteinuria shows variations between the two examinations similar to that of pyuria and of haematuria.

Proteinuria coexisted with pyuria in 11.3% on screening and in 15.2% at clinical examination.

Accordingly there is some correlation between the presence of pyuria and that of proteinuria, but factors other than pyuria can also explain the presence of proteinuria. An analysis for a correlation between varying degrees of pyuria and the presence of proteinuria gave uncertain results because of the relatively small number of specimens with positive protein reaction. The results confirm the well known experience that a protein test is insufficient as a means of diagnosis at screening for urinary tract infection and pyuria.

Diagnostic value of the Labstix test for haematuria

Table V shows the correlation between the number of RBC/HPF and the result of Labstix as a reagent to blood in the urine in 6183 specimens.

Using at least 3-7-10 and 20 red cells/HPF as criteria for haematuria, the incidence was

8.2% 3.4% 1.9% and 1.3% of the total number of examined specimens. If Labstix was considered positive at at least + the test showed blood in the urine in 15.4% 32.0% 46.0% and 28.0% respectively by the said criteria for haematuria. It will be seen from the Table that when the stricter criteria of +++ or ++++ were used, the number of positive tests decreased, that is, the number of false negatives increased.

Thus, the test was of no diagnostic value in demonstrating haematuria.

COMMENTS

The results have been summarized in the Abstract of this paper. The reader will find a discussion of important facts in connection with a survey of the literature in a subsequent paper (3), which will contain a report on the presence of white cells in urine obtained by percutaneous suprapubic aspiration from the bladder and by collection of clean-voided midstream specimens after the puncture from persons with and without bacteriuria. Results of methodological studies on the technique used by us in diagnosing pyuria will also be reported.

ACKNOWLEDGEMENTS

The study was supported by grants from Malmöhus Local Social Insurance Office and from AB Gambo, Lund.

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PYURIA. DEPOSIT IN HIGH POWER MICROSCOPIC FIELD—WBC/HPF— VERSUS WBC/mm³ IN COUNTING CHAMBER

Reappraisal of a Valuable Clinical Routine Method (Urinary Sediment)

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Abstract. The methodological study with defined techniques presented in this paper gave the result that the number of WBC/HPF in the sediment corresponded to approximately 11% of the number of cells/mm³ in the sample. The correlation appears to be satisfactory. Thus the criterion of >4 WBC/HPF for pyuria corresponds to roughly >40 cells/mm³ under the methodological conditions obtaining here.

Textbooks and manuals for medical students and doctors usually state, without any particular reason, a certain number of white cells per high-power microscopic field (WBC/HPF) as a diagnostic criterion—varying with different authors from a few to a large number—but the conditions under which the count is to be made, for instance the volume of urine in the centrifuge tube, the time of centrifugation and the r.p.m., are as a rule not given, the count is of ten made the criterion for a disease without any comparison with clinically healthy individuals. Pyuria is regarded as a "hallmark" of urinary tract infection and pyelonephritis—but insufficient for diagnosis.

The literature contains many criteria for pyuria, as a rule probably arbitrarily defined.

Criteria applied in literature. 3-5 WBC/HPF (12); at least 5 (6, 13, 14, 16); more than 10 (3, 4, 18) more than 20 (11) and "large number" (5). The volume of urine in the centrifuge tube, 5-12 ml, is normally only stated here: comparison is made between the number of WBC/HPF and per mm³ in the counting chamber. Only one or two authors give the duration and speed of centrifugation, e.g. 10 min at 4 000 r.p.m. (15), 3 or 5 min at 3 000 r.p.m. (4, 6). One author describes the subsequent procedure as follows: After centrifugation all the supernatant urine is discarded leaving 1 drop of sediment (5). When staining the sediment this volume is increased by the addition of, for instance, one drop of

Sternheimer-Malbin stain. It ought to be standard practice that the effect of this dilution on WBC/HPF is disregarded when stating the number of WBC/HPF.

Bearing in mind the many sources of error the WBC from the counting chamber has been used as a control. A problem arising here has been the criterion for pyuria expressed as the number of cells/mm³ unspun urine and the normal value for Addis count in clean-voided specimens from men and urine obtained by bladder catheterization in women.

Some examples. On the basis of an Addis count of max. 1.5 mill. WBC/24 hours one drop of sediment from 1 ml centrifuged urine is estimated to contain 7 200 cells, which corresponds to 3 WBC/HPF more than 5 WBC/HPF is defined as pyuria (17). Another author used the following normal value for an Addis count, 2 mill. white cells/300 ml corresponding to 20 cells/mm³ (7). Some textbooks set the upper limit for the Addis count at 4 mill. white cells, which would correspond to approximately 40 cells/mm³ urine. One textbook states more than 50 white cells per mm³ of unspun urine is suggestive of infection (6). Many "normally" found less than 10 cells/mm³ in catheter urine (15).

Comparison between the number of cells in the sediment of spun urine and the number per mm³ of unspun urine gave the result that 1 WBC/HPF corresponded to 5-6 cells/mm³ (15). Elsewhere (14) we find: "Uros, even though poor correlation was found between cell counts per HPF of centrifuged urine to cell counts per cubic millimeter of the same uncentrifuged sample, an overall correlation in the entire group was present" the criterion for pyuria was set at at least 5 WBC/HPF. "This definition enjoys wide acceptance in the United States and in Europe."

The present paper seems to be especially valuable for its account of essential methodological details, which are usually absent in the literature.

Mabock and Schjøtt-Christensen (9) published a comparison between the number of WBC in suspension urine seen in a Fuchs-Rosenthal counting chamber and in HPT in centrifuged stained sediment. 8 ml urine were centrifuged for 3 min at 1 500 r.p.m. The volume remaining in the tube after pouring off the supernatant is not given. One drop of stain was added. Since the distance between the cover slip and the slide, and thus the thickness of the sediment, varies, only those leucocytes which are in focus in the field of vision are counted. Ten fields were viewed with the largest dry objective. The diameter of the HPT is given as 0.30 mm and the depth of the sediment as approximately 0.025 mm. Ten fields were counted, the result being given as the maximum and minimum number of leucocytes per field. The leucocytes were stained with Sternheimer-Malbin stain. Only round, ovoid or granular cells of leucocyte size were counted. Pyuria was defined as the isolation of less than 400 000 leucocytes per hour corresponding to 10 or more mm^3 or 3 or more HPT. A diagram shows that the range of observations as large as 4 cases 0-3 WBC/HPT corresponded to at the most 10 WBC/ mm^3 to 8 cases to roughly 50 and in one case to about 100/ mm^3 . Between 4 and 5 WBC/HPT corresponded to two cases to at the most 10 leucocytes/ mm^3 and in 11 to higher values, of which the last between approximately 40 and 100 and one around 500. The uncertainty in the determination of WBC/HPT was also illustrated by the results of investigating ten samples of each of six urines. Uncertainty expressed as standard deviation and the coefficient of variation was also a feature of counting the leucocytes in the counting chamber.

METHOD

Details of the method are given below particularly where they deviate from those of the last mentioned authors.

The urine samples are noted carefully to ensure even distribution of their formed elements. Samples of 10 ml are placed in each of 8 centrifuge tubes of standard type. The samples are spun for 5 min at 3 000 r.p.m. The tubes are divided into two series and examined immediately by the following two methods.

a) Half the number of tubes were intended for determination of WBC/HPT. The supernatant urine was poured off by slowly tilting the tube until it reached an angle of approximately 90° to the horizontal. The last drop was allowed to run off spontaneously without shaking. One drop of Sternheimer-Malbin stain was then added using a drop pipette. The tube was carefully shaken so as to distribute the deposit evenly in the liquid. The final mixing was done with the aid of the pipette, whereupon the volume was measured. One drop as placed on a slide and covered with cover slip. For counting, a dry objective and 320 \times magnification were used. For each sediment 10 fields were counted, the micrometer screw being turned so to include 10 cells at various depths. In the following is the mean value for 10 fields is given.

b) Half of the tubes were intended for counting in the Bürker counting chamber. The supernatant was

poured off slowly leaving 1 ml in the tube. The volume was marked by a line. The liquid was stirred carefully. The number of cells in $1/10 \text{ mm}^3$ was counted. The result as the same both with and without addition of Sternheimer-Malbin stain, though the stain facilitated the counting.

The same urine specimen was examined in a Bürker chamber by a trained technician while another person (N.A.) determined the WBC/HPT. W worked independently of each other.

In a separate series the WBC/HPT was studied after the urine containing two drops of 10% thiomersal sodium solution to prevent bacterial growth, had been kept for 1-4 days at room temperature. These conditions were intended to match those for screening (1), when urine samples prepared in the manner mentioned are mailed to our laboratory.

RESULTS

After pouring off the supernatant urine as described above and adding one drop of Sternheimer-Malbin stain the centrifuge tube contained, in 75 consecutive samples, 0-3 ml (0.20-0.25); two-thirds of the values fell between 0.22-0-4 ml.

Fig. 1 shows 100 values for WBC/HPT each of which represents the mean value of the number of cells found in 10 microscopic fields and 100 values for WBC/ mm^3 in the same samples determined in a Bürker counting chamber. Four parallel determinations of WBC/HPT and WBC/ mm^3 were carried out on each of 25 urine samples. Ten samples contained <3 WBC/HPT, five 5-10 WBC/HPT, seven 11-20 WBC/HPT and three more than 20. The average number of WBC/HPT for the whole series was approximately 11% of the number of WBC/ mm^3 . The mean values for these diagnostic groups were as follows: <3 WBC/HPT corresponded to 11.0% (9.5-14.0) of the number of WBC/ mm^3 within the same group, 5-10 WBC/HPT 11.2% (9.7-12.3), 11-20 WBC/HPT 11.3% (10-12.8) and >20 WBC/HPT 10.3%. The last figure is mentioned with the reservation that the group contains only three samples.

When 20 urines with pH 5-6, WBC/HPT 11, 20 or >20 and the preservative added were kept at room temperature (20-22°C), the number of WBC/HPT remained constant for at least 3-4 days. Day 1 98.6% (92-103), day 2 99.8% (94-111), day 3 100.6% (95-106), day 4 90.1% (69-104). At pH 7 the number of WBC/HPT of 10 urines decreases slowly. Day 1 98.8%

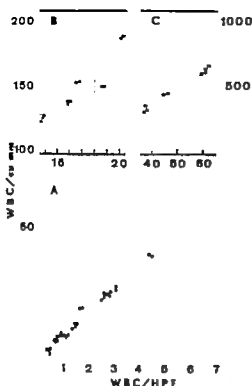


Fig. 1. WBC/HPF (A = <8 B = 13-21 C = 35-65) versus WBC/mm³.

(84-105), day 2, 95.2% (87-97) day 3, 86.4% (84-90) day 4, 82.1% (75-93).

As 71.1% of our screening samples had pH 5-6, 27.5% pH 7 and only 1.4% pH 8 and as the postal transport will seldom have lasted more than three days, these facts will not substantially influence the results of our screening (1).

COMMENTS

This study shows that determination of WBC/HPF can be a reliable test if special care is taken to ensure a volume as constant as possible in the centrifuge tube. This would seem to require a standardized technique for pouring off the supernatant, i.e. the deposit must be suspended in a relatively constant volume prior to viewing under the microscope. Since information of this kind is usually absent in the literature, comparison with other details about WBC/HPF particularly those stated as criteria for pyuria, cannot be made. The importance of methodological details of this nature, both for textbooks and

manuals for students and doctors and for scientific papers, must be stressed. Another important methodological detail is whether the WBC/HPF is counted only in one plane in the microscopic field (9) or whether as with the present technique the microscope micrometer screw is adjusted so as to include all the cells in the urine layer within the field of vision.

The number of WBC/HPF is approximately equivalent to 11% of the number of WBC/mm³ counted in the counting chamber. Agreement was good throughout, irrespective of the number of cells. If the criterion for pyuria is put at >4 WBC/HPF the criterion for pyuria expressed as the number of cells/mm³ will be roughly >40 WBC/mm³.

Tuxford (16) defined pyuria as a count of >50 WBC/mm³. Norden and Kass (12) found that only 50% of their bacteriuric patients had as many as 5 WBC/HPF and that lowering the value to 3 WBC/HPF added only 10% more with apparent pyuria.

In view of the results reported in this paper our population study (1), information in the literature quoted and general clinical experience, there would scarcely seem to be any reason for a lower criterion for pyuria than 5 or more WBC/HPF. The fact that the criterion was set at 3 or more WBC/HPF (9) may have been due to the number of cells having been counted only in one plane under the microscope.

This study does not deal with criteria for the excretion of a certain number of leucocytes per unit of time.

ACKNOWLEDGEMENTS

This study was supported by grants from Malmåsters Local Social Insurance Office and AB Östebro, Lund.

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A POPULATION STUDY ON RENAL AND URINARY TRACT DISEASES

III. A Diagnostic Dilemma. Occurrence of Pyuria and Bacteriuria in Conventional and Clean-voided Midstream Specimens and Suprapubic Urine

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Abstract. In a previous paper on a population study the prevalence of different degrees of pyuria in conventional urine samples and in clean-voided midstream specimens was reported. Discussion of the data collected was postponed until this publication, which contains a study of urines obtained by percutaneous suprapubic puncture of the bladder and clean-voided midstream specimens obtained at the same time. The present series comprises 155 persons in all, 113 of whom were men. 72 had significant bacteriuria in the two urines; 72 and 90% pyuria. For 83 persons with subsignificant bacteriuria or sterile urine the corresponding figures were 20 and 37%. Since the series comprised 76% women the sex factor cannot be estimated. Clean-voided midstream specimens reduced the occurrence of pyuria by about two-thirds in comparison to findings in conventional total voided samples. Percutaneous suprapubic aspiration of urine should be done particularly with the occurrence of pyuria without significant bacteriuria in clean-voided midstream specimens; in our series only in half the cases could the diagnosis be verified through pyuria also in suprapubic urine. There was no correlation between subsignificant bacteriuria and pyuria in midstream specimens. If confirmation is required in midstream specimens with significant bacteriuria, the agreement with corresponding findings in suprapubic urines was so good that they do not support general indications for aspiration of bladder urine under present conditions. A future paper will account for public health symptoms and clinical findings in the 155 patients who make up the series presented in this paper dealing with methodological problems.

In a previous paper on a population study (3) the prevalence of different degrees of pyuria in conventional urine samples and in clean-voided midstream specimens was reported. Discussion of the data collected was postponed until this publication, which contains a complementary study of urines obtained by percutaneous suprapubic

puncture of the bladder and clean-voided midstream specimens obtained at the same time. The results presented here concern a selection of patients who underwent treatment at the Renal Clinic in Lund during the years 1969-1971 without exception on the grounds of positive findings on screening and/or medical examination in connection with the population study. In only about two-thirds of the 155 cases in the series, however, did the findings of bacteriuria and/or pyuria indicate further examination.

METHODS AND MATERIAL

After centrifugation of the urine the number of white blood corpuscles per high power field (WBC/HPP) was counted using routine clinical method. The method was checked in a special study where the results of parallel investigations using this method and the counting chamber were compared (1). The method of sampling the urine was described in a previous paper (2). The patients had fasted and taken no fluid after 10 p.m. the previous evening. The interval between the last micturition and the percutaneous suprapubic aspiration was 10 hours. Clean-voided midstream specimens were obtained about 1/2 hour after the puncture. The urine cultures were carried out at the Department of Microbiology of the University Hospital. The results for 141 persons included in this series were reported in a previous paper (2).

The occurrence of different types of *Mycoplasma* was reported by Mårdh et al. (3).

The series comprises 155 persons in all, 113 of whom are men (73.8%); 72 (46.5%) had significant bacteriuria in both suprapubic urine and voided specimens. In the remainder the samples were sterile or displayed only sub-significant growth of bacteria. One voided specimen, however, showed significant bacteriuria.

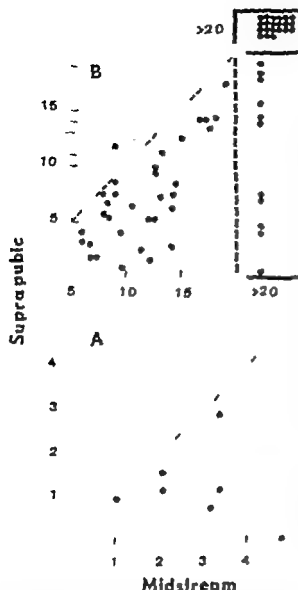


Fig. 1. Observation of WBC/HPF in 17 patients with significant bacteriuria in suprapubic urine and midstream specimens. Data from men (with at least 5 WBC/HPF in midstream specimen) (B) and cases with values below 5 in the two samples (A).

RESULTS

Seventy-two persons, including 7 men, displayed significant bacteriuria in both suprapubic urine and voided specimen (Fig. 1). Significant bacteriuria (over 10^5 bacteria/ml) was caused in 95.6% by *E. coli* the sensitivity to antibiotics, etc. similar in both samples. In the remaining cases there were coliform rods, *Enterococci*, etc. A description of other details would seem to be beyond the scope of this paper.

Table I shows the WBC/HPF count for the same diagnostic groups as in the preceding work (3): <5, 5-10, 11-20 and >20. The distribution of the subjects was as follows: over 60 years 21 (4 men), 51-60 years 23 (3 men) and <50 years 28 (0 men). Within these age groups there was (a) a WBC/HPF count of at least 5 in suprapubic urines and midstream specimens in 76.2 and 76.2, 73.9 and 87.0% and 71.4 and 71.4% respectively; thus the findings in the two samples showed good agreement in all the age groups; (b) a WBC/HPF count of at least 11 in 61.0 and 71.4, 34.8 and 69.6% and 32.1 and 60.7% respectively. Thus the frequency of this degree of pyuria was higher in voided specimens than in suprapubic urines in all the age groups, especially the lower ones.

A WBC/HPF count of less than 5 was found in 77.5% of the suprapubic urines and in 10.1% of the voided specimens. Thus at this upper normal limit for WBC/HPF pyuria occurred in 72.5 and 89.9% respectively in the two samples. If the upper limit is set at 11 WBC/HPF these values for pyuria are 50.0 and 69.4%.

Only 9.7% of the cases examined were men, so no conclusions may be drawn with regard to possible differences between the sexes.

Table I. WBC/HPF count of suprapubic urine and midstream specimens from patients with significant bacteriuria in the two samples

Figures within parentheses indicate men

Age (y)	Suprapubic urine	Clean-voided midstream specimens				Total
		<5	5-10	11-20	>20	
>60	<5	—	1	1	1	3
	5-10	—	—	2 (1)	1	3 (1)
	11-20	—	—	—	3	3
	>20	—	—	—	10 (3)	10 (3)
	Total	2	1	3 (1)	15 (3)	21 (4)
51-60	<5	2	2	2	—	6
	5-10	—	1	2	—	3
	11-20	1 (1)	1 (1)	5	2 (1)	9 (3)
	>20	—	—	—	5	5
	Total	3 (1)	4 (1)	9	7 (1)	23 (3)
<50	<5	3	4	—	1	8
	5-10	—	5	5	1	11
	11-20	—	—	2	1	3
	>20	—	—	—	6	6
	Total	3	9	7	8	27
Total		8 (1)	14 (1)	19 (1)	31 (4)	72 (7)

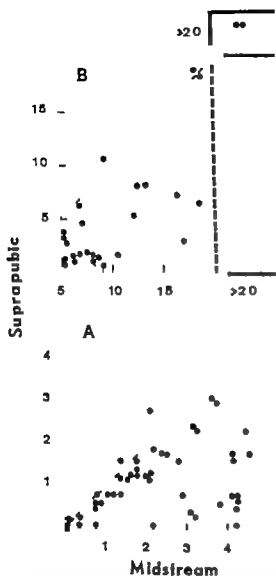


Fig. 2 Observations of WBC/HPF in 82 cases with sterile urine or subsignificant bacteriuria in suprapubic and midsream specimens plus one case with significant bacteriuria in the latter sample alone. Data from cases with at least 5 WBC/HPF in midsream specimens (B) and cases with values below 5 in the two samples (A).

Eighty-three persons, including 19 men, (Fig. 2) displayed (a) suprapubic urine sterile in 76 cases, bacterial growth $<10^4$ /ml in one case and 10^4 – 10^5 in six cases; (b) voided specimen sterile in 26 cases, bacterial count 10^4 /ml in 24 cases, 10^4 – 10^5 in 32 cases and 10^5 in one case. There follows an account of the number of WBC/HPF for cases in this series which did not have significant

bacteriuria in suprapubic urine. The results of the urine cultures will then be placed in relation to the sediment findings of the two urine samples.

Table II shows WBC/HPF counts in the diagnostic groups: <5 5–10 11–20 and >20 respectively. The distribution with regard to age group and sex was as follows: over 60, 17 cases (1 man); 51–60 26 (7 men, 27%) 21–50 79 (11 38%). Within these age groups the following WBC/HPF counts were found in suprapubic urines and voided specimens, respectively: (a) at least 5 WBC/HPF in 11.8 and 47.1% 23.1 and 30.8% and 20.0 and 37.5% the number of WBC was thus higher percentage-wise in voided specimens, especially in the highest age group (b) >10 WBC in 0 and 5.9% 23.1 and 30.8% and 10.0 and 12.5% thus there is hardly any difference in percentages between the WBC/HPF counts in the two samples.

Less than 5 WBC/HPF occurred in 79.5% of the suprapubic urines and in 62.7% of the voided specimens. Thus at the upper normal limit for WBC pyuria occurred in the two samples in 20.5 and 37.3% respectively. If the upper limit is set at 11 WBC/HPF these values for pyuria are 7.2 and 13.3%.

Table II. WBC/HPF count of suprapubic urine and midsream specimens from patients with sterile urine and/or subsignificant bacteriuria, with exception of one midsream specimen with significant bacteriuria

Figures within parentheses indicate sex.

Age (y)	Suprapubic urine	Clean-voided midsream specimens				
		<5	5–10	11–20	≥ 20	Total
>60	<5	9 (1)	6	—	—	15 (1)
	5–10	—	1	—	1	2
	11–20	—	—	—	—	—
	>20	—	—	—	—	—
	Total	9 (1)	7	—	1	17 (1)
51–60	<5	18 (2)	2	—	—	20 (2)
	5–10	—	2 (1)	3 (3)	—	5 (4)
	11–20	—	—	—	—	—
	>20	—	—	—	1 (1)	1 (1)
	Total	18 (2)	4 (1)	3 (3)	1 (1)	26 (7)
21–50	<5	25 (5)	7 (1)	—	—	32 (6)
	5–10	—	2 (1)	2 (2)	—	4 (3)
	11–20	—	1	2 (1)	—	3 (1)
	>20	—	—	—	1 (1)	1 (1)
	Total	25 (5)	10 (2)	4 (3)	1 (1)	40 (11)
Total		52 (8)	21 (3)	7 (6)	2 (2)	83 (19)

The comparatively small number of men in the series renders it impossible to make a definite comparison between the sexes. Despite this, a few figures will be given.

Less than 5 WBC/HPF in suprapubic urine and voided specimen occurred in 47.4 and 42.1% of the men, respectively; at least 5 WBC/HPF in 52.6 and 57.9% at least 11 WBC/HPF in 15.9 and 42.1% of the men. The percentages are given with the reservation that the number of observations was relatively small, the difference found between the higher frequency of severe pyuria in voided specimens than in suprapubic urines with the severest degree of pyuria is not definite. The corresponding values for the 64 women were less than 5 WBC/HPF in 90.6 and 68.8% at least 5 in 9.4 and 31.2% at least 11 in 11 and 31.2% respectively.

The following is a presentation of the results of urinary cultures and the findings in urinary sediments in 82 cases where no significant bacterial growth ($>10^5$ /ml) was found in either suprapubic urine or voided specimen and one case which displayed significant bacteriuria in the voided specimen.

(a) Suprapubic urine in cases with 10^4 – 10^5 E. coli/ml. Voided specimen one sample as sterile and one showed significant growth of E. coli. The latter sample alone displayed pyuria (16.8 WBC/HPF); the rest had <5 WBC/HPF.

(b) Suprapubic urine four cases with 10^4 – 10^5 Enterococci. Voided specimen similar growth. While the remainder had <5 WBC/HPF pyuria as found in both samples in one case (11.8 and 19.7 respectively) and in voided specimen alone in one case (>2 WBC/HPF).

(c) Suprapubic urine one case with $<10^4$ Enterococci. Voided specimen 10^4 – 10^5 Enterococci. No pyuria.

(d) Ten sterile cases with sterile suprapubic urines and 10^4 – 10^5 bacteria/ml in the voided specimen. Enterococci 10%, diptheroids 27%, coagulase negative Staphylococci 11% and mixed infections 52%. Only voided specimens displayed pyuria: 1 least 4 WBC/HPF in nine cases and more than 10 WBC/HPF in one of them. No correlation between bacterial growth and pyuria.

(e) Twenty-four cases with sterile suprapubic urine and $<10^4$ bacteria/ml in the voided specimen. Enterococci 33%, coagulase negative Staphylococci 1%, diptheroids 6%, E. coli 4% and mixed infections 54%. Six cases had pyuria in both samples, and one case had it in the voided specimen alone. The material does not permit conclusions to be drawn as to the correlation between bacterial growth and pyuria.

Owing to too few observations under points a–e the results of the Mycoplasma cultures, which in most cases are negative, are not given.

(f) Twenty-five cases with sterile suprapubic urine and

voided specimen. <5 WBC/HPF occurred in both samples in 21 and 18 cases, respectively. 5–10 in 3 and 4 cases, and 11–20 in 2 and 3 cases, respectively. The mean values for WBC/HPF in the two samples are 2.6 (0–12.3) and 2.2 (0.4–17.2), respectively.

In 10 of the 25 cases mentioned no examination was made with respect to the occurrence of Mycoplasma spp. The number of WBC/HPF averaged 2.7 (0.6–12.3) in suprapubic urines and 4.7 (0.9–17.2) in voided specimens; <5 WBC/HPF occurred in 9 and 6 cases.

Ten of the cases, of whom nine are women, displayed negative Mycoplasma cultures and had pyuria in neither sample: 0.6 (0–1.4) and 2.2 (0.4–4.2) WBC/HPF respectively. The remaining five cases: one case its suprapubic culture in suprapubic urine but no examination of the voided specimen had pyuria in both samples (5.3 and 1.2 WBC/HPF respectively); one case displayed Mycoplasma and pyuria in both samples (10.6–9.4) and three cases had negative cultures in suprapubic urine and positive in voided specimen, one of these having pyuria in both samples (8.2 and 1.6 WBC/HPF respectively).

Summing up the following WBC/HPF counts were found in the first series with significant bacteriuria, 72 cases, and in the remaining series, 83 cases (and in 25 selected cases from this latter series with both samples sterile): <5 WBC/HPF in suprapubic urine 4 and 80% (84%) and in voided specimen 10 and 88% (77%); ≥ 5 WBC/HPF 76 and 20% (16%) and 90 and 37% (28%); ≥ 11 WBC/HPF 50 and 7% (8%) and 69 and 13 (1%). Thus pyuria— ≥ 5 WBC/HPF—occurs in higher frequency with significant bacteriuria in both samples than in other cases. The frequency is probably lowest if both samples are sterile, particularly if Mycoplasma spp. is also absent. Voided specimens have higher frequency of pyuria than suprapubic urines. The frequency of pyuria in voided specimens does not appear to increase in the presence of subsignificant bacterial growth (10^4 – 10^5 or $<10^4$ /ml). The patients examined were not treated with antibiotics or other drugs, so the results have not been affected by therapy of this kind.

It is beyond the scope of this paper to give a closer clinical account of the cases which showed subsignificant growth of bacteria in suprapubic urine although this symptom is considered to be pathological.

COMMENT

The following discussion is confined chiefly to technical problems of practical importance in clinical routine. The results mentioned are taken

partly from the present investigation of suprapubic urine and subsequent clean-voided midstream specimen in 155 selected patients and partly from a population study reported in a preceding paper which comprised screening of 3 998 persons and medical examination of 2 276 of them (3). The discussion concerns, among other things, the techniques for urine sampling—conventional urine samples, clean-voided midstream specimens and suprapubic urines.

In a previous paper (3) it was reported that owing to a departure from the planned investigation the screening of the first 1 255 persons was done on conventional urine samples. Subsequently clean-voided midstream specimens from 2 643 individuals were examined. The latter series showed on average about 60% lower frequency of pyuria than the former irrespective of the criteria applied, at least 5 at least 11 and at least 21 WBC/HPF. Midstream specimens revealed pyuria (at least 5 WBC/HPF) in on an average 3.2% of the men and 17.3% of the women. If the criteria at least 11 and at least 21 WBC/HPF were applied, pyuria was found in 1.6 and 0.5% and 10.0 and 3.7% respectively. For men the figures were highest in the oldest and youngest age groups and lowest in the 41–50 age group. Others have also found a relatively high frequency of pyuria in young men (15). That the prevalence of pyuria is greater in women than in men is well known. For women the frequency of pyuria broadly speaking increases with age, irrespective of the method of urine sampling or the criteria for pyuria.

This paper presents a study of pyuria frequency in 72 persons with significant bacteriuria in both suprapubic urine and subsequent midstream specimen: 72 and 90% pyuria, that is to say about 20% less for suprapubic urine than for midstream specimens. For 83 persons with sub-significant bacteriuria or sterile urine the corresponding figures were 20 and 37%—a difference of almost 50%. Since the series comprised 76% women the sex factor cannot be estimated.

Mabeck (8) found an insignificantly higher number of leucocytes in midstream specimens than in suprapubic urine ("good agreement"). Wiebel et al. (16) noted a great number of WBC/HPF ("massenhaft Leukoryten") in on an average 3.5 and 8% respectively of the two samples in 215 children, in cases with sterile bladder urine

the difference was slight. Berchtold et al. (5), who defined pyuria as >10 WBC/HPF noted 26.2% "falsely positive" cases of pyuria and bacteriuria in midstream specimens in 521 patients with clear suprapubic urine.

It might appear unnecessary to repeat that clean-voided midstream specimens reduce the occurrence of pyuria by about two-thirds in comparison to findings in conventional total voided samples. Yet this latter method is still widely used both in medical examination (WBC/HPF and Addis count) and in screening. Percutaneous suprapubic aspiration of urine should be done particularly with the occurrence of pyuria without significant bacteriuria in clean-voided midstream specimens. Only in half the cases in our material could the diagnosis be clarified through pyuria also in suprapubic urine.

It is well known that pyuria can appear intermittently in voided urine. The diagnostic problem is illustrated by our figures (3): out of 336 persons with pyuria (>4 WBC/HPF), on screening and/or subsequent medical examination revealed pyuria in scarcely one-third, screening alone in fully one third and medical examination alone in one-third. The extent to which pyuria in suprapubic urine is also intermittent has yet to be investigated.

The series of 155 patients presented here, whose suprapubic urine and midstream specimens were investigated, comprises a selected material. For this reason it is not possible to make direct comparisons with other published material, particularly pregnant women and children, with regard to the occurrence of bacteriuria and pyuria. Of our cases 72 had significant bacteriuria in both suprapubic urine and voided specimens, the infection was caused by *E. coli* in 95.6%. In addition one case had 10^4 – 10^5 /ml *E. coli* in suprapubic urine and more than 10^6 in voided specimen. Pyuria (>4 WBC/HPF) occurred in 74% in suprapubic urine and in 89% in midstream specimens. Only 38% of 76 sterile suprapubic urines from our remaining cases displayed pyuria. The findings in voided midstream specimens are discussed below. Patients were not given antibiotics or other drugs for urinary tract infections. With regard to *L-forms* and *Mycoplasma* spp. the investigation has only been carried out to a limited extent. The results will not be discussed in respect of infections of this type. Since a

terial count of 10^4 – 10^5 /ml in the bladder urine which occurred in one instance is considered to be a sign of infection there was 100% agreement between significant bacteriuria in suprapubic urine and the corresponding infection in midstream urine. It is true that pyuria occurs to a greater extent with significant bacteriuria than when the urine is sterile but in our material there was no correlation between subsignificant bacteriuria and pyuria in midstream specimens. The results are based on only one study. Since both bacteriuria and pyuria could be intermittent, varying during the day and from day to day (7, 11) repeated studies might well provide a better basis for judging a possible bacteriuria-pyuria correlation in suprapubic urines and clean-voided specimens.

Another important factor which we have not investigated is the localization of the infection. In 40% of a series of 85 women with bacteriuria in suprapubic specimens the infection was confined to the bladder (13). Other authors have done valuable research on the localization of urinary tract infections through instrumentation (6, 7, 12). A recently evolved technique is the water-loading test (10).

In contrast to children (4, 17) the determination of antibody response in urinary tract infections in adults does not appear to be definitely related to the localization (14). We have been unable to undertake studies of this kind.

The diagnosis of bacteriuria is complicated by the possibility that bacteriophages or other lytic agents, such as colicins, may cause apparent sterile pyuria (7).

Well known traumatic causes of pyuria, such as instrumentation of the urinary tract or stones, do not affect our results, neither do the misuse of analgetics or renal papillary necrosis. We diagnosed only two cases of urinary tract tuberculosis in the screened persons (3). Neither of these cases is included in the present material.

Among our 82 cases, which displayed either sterile specimens or subsignificant bacteriuria in suprapubic urine and/or midstream specimen, five cases with a bacterial count of 10^4 – 10^5 /ml were noticed, of which one was *E. coli* and the rest were *Enterococci*. The corresponding midstream specimens revealed similar counts and the same bacteria, with the exception of one sterile sample (the case with *E. coli*). One suprapubic

sample with $<10^4$ *Enterococci* had a correspondingly higher number 10^4 – 10^5 . Sterile suprapubic urine in 76 patients had corresponding sterile midstream specimens in 25 cases, a bacterial count of $<10^4$ in 24 cases and a count of 10^4 – 10^5 mainly *Enterococci* diphtheroids, etc., in 27 cases. Although the subsignificant bacteriuria in midstream specimens can be caused by contamination, the findings brought about the following comments as a result of a remark in the literature that 98% of women with sterile urine in the bladder cannot produce a midstream specimen free of bacteria, even under the best conditions between 10 and 20% of midstream urines from women contain 100 000 or more bacteria per ml. levels at which infection is usually judged to be present" (13). With the reservation that our material contains fewer men than women it may be pointed out that roughly one-third of the cases of both sexes with sterile bladder urine also had sterile midstream specimens. The percentage of cases with $<10^4$ and 10^4 – 10^5 bacteria/ml also showed reasonable agreement for both sexes.

It would seem, then, that we have been able to achieve satisfactory diagnostic results with our technique for clean-voided midstream specimens, even though the sampling has been carried out by the patients themselves. If we confine ourselves to midstream specimens with significant bacteriuria, the agreement with findings of bacteria in suprapubic urine is so good that they do not support the indications for aspiration of bladder urine under present conditions. In the case of pyuria, the findings in suprapubic urines appear to have been of considerable diagnostic significance through the reduction of the pyuria frequency.

A future work will account for subjective symptoms and clinical findings in the 155 patients who make up the series presented in this paper.

ACKNOWLEDGEMENT

This study was supported by grants from Malmohus Local Social Insurance Office and AB Gambro, Lund.

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INITIAL CLINICAL EXPERIENCE WITH I.C.I. 66.082, A NEW β -ADRENERGIC BLOCKING AGENT IN HYPERTENSION

PRELIMINARY REPORT

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I.C.I. 66.082, 4-(2-hydroxy 3-isopropylamino-propoxy)phenylacetamide, is a new β -adrenergic blocking agent. Initial animal studies have shown this drug to be a specific inhibitor of cardiac (β 1) β -receptors. Furthermore, it does not readily cross the blood/brain barrier or possess intrinsic sympathomimetic properties or membrane stabilizing effects. Thus, this agent would seem useful in the treatment of hypertension and could theoretically offer advantages over currently used β -adrenergic blockers.

MATERIAL AND METHODS

In multicenter trial, I.C.I. 66.082 was given to 20 hospitalized patients, 12 men and 8 women, with essential hypertension, whose average age was 43 years (range 23-57). Fundoscopic examination revealed normal fundi in 4 patients, degree I changes in 8 and degree II in 8. Two patients had left ventricular hypertrophy radiologically. No patient received antihypertensive therapy prior to the present trial, nor were any other drugs given during the study. Treatment was started after a few days of hospitalization. Initially 30 mg I.C.I. 66.082 was given twice daily but the dosage was increased to 100 mg twice daily during days 2-7. Blood pressure (BP) and heart rate (HR) were recorded at least 4 times daily after 10 min of recumbency and 2 min of standing. A number of hematological and metabolic tests were made before and during active therapy (Table I). Laboratory test results, BP and HR were analysed statistically (paired *t*-test) by comparison of data from the last untreated day to those obtained during day 7 of active therapy.

RESULTS

No significant changes of hematological or metabolic parameters were observed (Table I). In addition

to the tests shown in Table I, differential count of white blood cells and urine microscopy were performed, both without consistent changes. Average weight was 76.8 kg before and 77.0 kg during treatment.

Significant reductions of HR and BP occurred (Table II). In one patient, a woman aged 51 treatment was withdrawn after 3 days due to sinus bradycardia (45 beats/min). Data from this patient have not been included in the calculations. Aside from this, no side-effects were observed during the trial.

DISCUSSION

Beta-adrenergic blocking agents have been used successfully in antihypertensive therapy for almost a decade (1, 2, 3, 4). One reason for the

Table I. Laboratory parameters

	Before	After	Difference	Significance
Hb (g/100 ml)	14.9	14.4	0.5	n.s.
WBC/cd	6 300	6 200	100	1
Creatinine/s (mg/100 ml)	0.9	0.9	—	1
Uric acid/s (mg/100 ml)	5.5	5.8	0.3	1
SGOT (U/l)	15.0	14.0	1.0	1
SGPT (U/l)	15.6	14.5	1.1	n.s.
Bilirubin/s (mg/100 ml)	0.7	0.6	0.1	1
Fasting blood sugar (mg/100 ml)	86.4	88.1	1.7	n.s.

Table II. *Changes of blood pressure (mmHg) and heart rate (beats/min) (mean \pm S.E.M.)*

	Recumbent		Standing		Recumbent HR
	BP _s	BP _d	BP _s	BP _d	
Before	169.5 \pm 4.0	105.3 \pm 2.0	171.8 \pm 4.5	112.1 \pm 1.7	77.2 \pm 2.2
After	140.5 \pm 4.3	87.9 \pm 3.0	142.4 \pm 4.5	92.4 \pm 2.5	61.3 \pm 1.4
Difference	28.9 \pm 5.0	17.4 \pm 3.3	29.5 \pm 4.8	19.7 \pm 3.7	15.8 \pm 2.8
Significance	$p < 0.0005$	$p < 0.0005$	$p < 0.0005$	$p < 0.0005$	$p < 0.0005$

increasing use of these agents in hypertension has been their relative lack of serious side-effects. However in large series a certain number of patients seem to develop bronchoconstriction while others complain of fatigue vivid dreams, sleep disturbances or even hallucinations undoubtedly due to central nervous effects (1-4). For these reasons, a β -adrenergic blocking agent without effects on smooth muscles of the bronchi or CNS effects would be very useful.

The present study of a new drug with the desired properties in these respects was directed primarily to the study of metabolic effects, as I.C.I. 66.082, so far has been used only in animal research. During the limited period of observation no effects on hematological or metabolic parameters occurred, which is in full agreement with the initial animal studies. Interestingly a marked hypotensive effect was observed even during the first week of therapy. Although reduction of BP is a common finding in hospitalized patients with hypertension, the observed changes are certainly greater than would be expected from hospitalization as such. Clearly this finding mer-

its further study on an out-patient basis and preliminary results from an ongoing study confirm that I.C.I. 66.082 has a useful antihypertensive effect.

Our conclusions therefore are that the lack of metabolic effects and serious side-effects, as well as the observed antihypertensive effect of I.C.I. 66.082, merit further study of this interesting new compound which may prove to offer advantages over currently used β -adrenergic blocking agents.

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TRYPTOPHAN CONCENTRATION IN SERUM OF PATIENTS WITH REGIONAL ENTERITIS

Its Possible Relation to Depression

PRELIMINARY REPORT

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In recent years much interest has been focused on tryptophan as a precursor to active metabolites like 5-hydroxytryptamine (serotonin) and tryptamine. It has been suggested that a deficiency of serotonin in the brain might be a pathogenetic factor in certain depressive states. One reason for such a deficiency is a low tryptophan pool, caused either by an increased need of tryptophan or by a changed metabolism or by a defect absorption from the intestine.

Lehmann (7) recently correlated mental symptoms to tryptophan deficiency in patients with the carcinoid syndrome. The amino acid has been tried in the treatment of depressions since the report by Oates and Sjoerdsma (8) that L-tryptophan potentiated the clinical response to monoamineoxidase inhibitors. In a group of patients with depression the serum level of S-tryptophan was found to be mainly within normal limits (1).

Although the serum levels of tryptophan have not been determined in patients with malabsorptive disorders, an increased urinary excretion of indican has been demonstrated in non-tropical sprue (5), jejunal diverticulosis, pancreatic insufficiency and regional enteritis (3). This indicates a defect absorption of tryptophan, which might result in a decreased tryptophan pool.

Evidently several factors may contribute to the depressive states not infrequently found in patients with malabsorption. Considering the findings mentioned above, it seemed of interest to determine serum tryptophan in a patient group liable to malabsorption and to examine its possible relationship to depressive states.

MATERIAL AND METHODS

Twenty patients with regional enteritis were chosen for pilot study. In 17 the diagnosis was confirmed histologically in three by X-ray examination only. The patients were continuously supervised by doctors trained to diagnose psychosomatic and psychic disorders. Fasting serum levels of S-tryptophan are determined by specific fluorimetric method (2), modified according to Lehmann (6), giving normal values between 44 and 78 $\mu\text{mol/l}$. S-tyrosine in serum is determined according to Udenfriend and Cooper (9). The possible presence of vitamin B₆ deficiency was evaluated by determination of S-GOT in serum before and after addition of pyridoxal-5-phosphate (Py-5-P) (4).

RESULTS

The results together with some clinical data are given in Table I. The value for serum S-tyrosine was above 50 $\mu\text{mol/l}$ and the quotient S-GOT + Py-5-P over S-GOT was below 1.5 in all patients, i.e. normal findings.

Very low S-tryptophan values were found in three patients who had recurrent disease with intestinal stenosis and steatorrhea after ileal resections. Subnormal values were found in two patients (cases 4-6) with intestinal stenosis and increased fecal fat excretion, and in three others without stenosis. Of these last three, one (case 5) had a generalized amyloid reaction, and another (case 7) had undergone a large intestinal resection. Of 12 patients with normal S-tryptophan values four had ileostomies, three of whom after extensive ileal resections. A considerable fecal fat excretion was found in two of these patients (simultaneous determination of fecal fat in mis-

Table I. Fasting levels of serum S-tryptophan in 20 patients with regional enteritis (normal range 44–78 $\mu\text{mol/l}$)

Case no.	Serum S-tryptophan ($\mu\text{mol/l}$)	Type of operation and anastomosis	Length of resected fical segment (cm)	Presence of intestinal stenosis	Fecal loss of Ist (Normally <7) (g/24 h)
1	0.3	By-pass	100	+	40
2	0.45	Ileo-transv	120	+	25
3	0.46	Ileo-desc.	60	+	20
4	96	Ileo-transv	50	+	16
5	30	Ileo-transv	60	—	7
6	18	None		+	13
7	0.41	Ileo desc.	100	—	6
8	0.48	Ileo rectal	10	—	5
9	0.48	None		—	6
10	0.38	Ileostomy	130	—	30
11	54	Ileostomy	25	—	4
12	0.45	Ileostomy	100	—	—
13	30	None		—	7
14	16	Ileo rectal	40	—	6
15	30	None		—	5
16	0.46	Ileo rectal	20	—	—
17	0.45	None		—	5
18	0.41	None		—	4
19	0.45	Ileostomy	130	—	30
20	35	None			6

ing for case 12). No patient with normal value of S-tryptophan had intestinal stenosis.

In the group of 20 patients, depression had been diagnosed in four (cases 1, 3, 4 and 6). Previous treatment with tricyclic antidepressants to these four had given no or only moderate relief of depressive symptoms. Very low values for serum tryptophan were found in two of these patients and lowered values in the other two. Three patients (nos 1, 3 and 6) reported improvement of depression and increased working ability during treatment for long periods with 0.6–1.2 g L-tryptophan/day (capsules containing 0.2 g). Their fasting S-tryptophan levels had by then been normalized. Withdrawal of the medication has been tried by the three patients, but return of depressive symptoms prompted them to resume treatment. In case 4 the treatment could not be tried since the patient had difficulty in swallowing the capsules.

These results indicate that a lowered or sometimes very low serum level of S-tryptophan in regional enteritis can be expected in patients with intestinal stenosis and steatorrhea. The occurrence of depression in patients with low S-tryptophan values and the improvement of the depression during the administration of L-tryptophan seem to indicate a possible relationship between

a low serum tryptophan level and depressive states. A controlled study is in progress to analyse further this possible relationship.

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CONCENTRATION OF CHOLESTEROL AND TRIGLYCERIDES IN SKELETAL MUSCLE OF HEALTHY MEN AND MYOCARDIAL INFARCTION PATIENTS

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Abstract. The concentration of cholesterol and triglycerides (TG) has been determined in muscle taken by needle biopsy from 47 healthy men, 50 male myocardial infarction (MI) patients and 21 50-year-old men with hypertriglyceridaemia (HTL) as the only sign. In healthy men the cholesterol concentration in muscle increased with age ($r=0.48$, $p<0.001$). The concentration of TG in muscle as weakly correlated to the concentration of TG in plasma ($r=0.30$, $p<0.05$) but not to age. The muscle TG concentration in HTL subjects was of the same order of magnitude as in healthy men. MI patients had a higher concentration of cholesterol in muscle than healthy subjects. Also the TG concentration in muscle was higher in coronary patients. In neither healthy men ($r=0.04$, $p>0.05$) nor MI patients ($r=0.13$, $p>0.05$) was the cholesterol concentration in muscle correlated to the cholesterol concentration in plasma.

In man little information is available on the metabolism of muscle lipids or on their behaviour during ageing and in different clinical conditions. Evidence has, however, accumulated from studies *in vivo* (9, 11, 39) and *in vitro* (1, 17, 32) suggesting that the metabolism of muscle triglycerides (TG) is related to the metabolism of plasma free fatty acids (FFA). The relationship recently reported between the concentration of TG in plasma and muscle (20) does not exclude that muscle tissue may be of metabolic significance also for the homeostasis of plasma TG. This view is further supported by the lower TG concentration in plasma and muscle after acute exercise (21) as well as physical training (8).

We have previously demonstrated an increase in the concentration of TG and cholesterol in muscle of rat with advancing age (10). A similar increase in the cholesterol concentration has been reported for muscle obtained in some autopsy materials (14, 27) but not all (16, 25, 31, 33). In this study further results are presented on the

concentration of TG as well as of cholesterol in muscle obtained by needle biopsy from healthy men of different age.

Data are also given on the concentration of cholesterol and TG in muscle for myocardial infarction (MI) patients, as this disease is associated with increased cholesterol accumulation in the arteries (13) and high incidence of hyperlipidaemia (7, 12, 18, 30). Maurizi et al. (31) reported low values for the cholesterol concentration in muscle in an autopsy material with arteriosclerotic vascular disease. Holm et al. (24) found no difference in the lipid concentration of leg muscle from subjects with and without arterial insufficiency. Since, however, metabolic adaptation to, e.g., hypoxia may have influenced the lipid values in muscle from these patients, it was of interest to determine the level of cholesterol and TG in muscle in another group of patients, also with advanced arteriosclerotic vascular disease, which, however, did not per se reduce the blood flow to muscle.

SUBJECTS

Group 1 consisted of 47 male volunteers, 30-72 years of age. They were randomly selected from participants in a health survey study then in progress, who in turn had been randomly selected from the city of Uppsala (19). All had normal laboratory data and clinical history. At the time for muscle biopsy none complained of physical discomfort or took drugs of any kind. None participated in competitive sport activities. These subjects are referred to as controls.

Group 2, MI patients, consisted of 50 men, 37-61 years of age. All had been hospitalized for acute myocardial infarction 3 months before blood and tissue sampling was done. The majority of these patients were on permanent drug therapy usually digitalis, in some cases also β -blocking drugs. No previous advice

Table I. *Antropometric data of control subjects, MI patients and HL subjects (mean \pm S.E.M., range given within parentheses)*

No. of subjects	Height (cm)	Weight (kg)	Index
Controls 47	176 \pm 0.9 (164-189)	75.3 \pm 1.2 (61-107)	1.0 \pm 0.1 (0.83-1.25)
MI 50	171 \pm 0.8 (164-195)	73.6 \pm 1.3 (54-95)	1.02 \pm 0.02 (0.74-1.33)
HL 21	174 \pm 1.3 (165-192)	75.0 \pm 2.1 (57-95)	1.01 \pm 0.02 (0.87-1.27)

Weight/Height = 100.

regard to plasma lipid lowering regimes, exercise training or weight reduction had been given. None had any symptoms of heart insufficiency. Patients with clinical diabetes (blood glucose concentration exceeding 110 mg%, glycosuria or antidiabetic treatment) were excluded from the study.

Group 3 HL subjects, consisted of 190-year-old men with hypertriglyceridaemia (HL). These subjects were participating in another health survey of 50-year-old men in the city of Uppsala and had elevated plasma lipid levels as only sign. All these subjects were on full-time work, did not complain of physical discomfort and did not take drugs of any kind. No advice had been given.

In regard to diet Table I give some antropometric data of the subjects in the three groups.

METHODS

Sampling done after overnight fasting during Nov-June 1971. Blood for determination of plasma lipids was drawn in an airtight glass and collected in plastic syringes, heparin not used. The muscle biopsy was taken in the belly of the vastus lateralis femoris (3) midway between the trochanter major and the proximal, lateral part of the patella. To skin as locally anaesthetized (0.4 ml 1% lidocaine, Astra Södertälje, Sweden) and an incision in the skin, about 3 mm, was done to facilitate the introduction of the biopsy needle.

The muscle tissue obtained in the biopsy needle was immediately frozen and stored frozen at -15°C . When further processed the muscle tissue was thawed by immersion into 0.5-1 ml 1% merthiolate in saline at room temperature. Adipose tissue was removed by dissecting the muscle tissue into 40 under a dissecting microscope. The thawing of the muscle tissue took ~ 3 min, the dissection about 1 min. The muscle material was then refrozen in liquid nitrogen, freeze-dried and divided into 4-6 parts for separate lipid determination. The weight of the tissue aliquots, 3-6 mg dry weight, as determined on an electromagnetic balance.

Extraction of lipids was done by homogenizing the muscle tissue with 1 ml methanol, 1 ml chloroform was then added followed by 3 ml saline. After overnight

equilibration the chloroform phase was transferred to a centrifuge tube. The supernatant remaining in the homogenizer was washed twice with 1 ml chloroform. The washes were combined with the initial chloroform phase in the centrifuge tube. After evaporating the chloroform the lipid residue was dissolved in 1 ml isopropyl alcohol. The phospholipids were removed by silicic acid added to the isopropyl alcohol. The concentration of TG and cholesterol in the isopropyl alcohol solvent was then determined by the Autoanalyzer technique as described for determination of cholesterol and TG in plasma (79): 0.5 ml 1% Vylocan 2 or 0.5 ml merthiolate solutions did not influence isopropyl alcohol blank readings in the Autoanalyzer.

All values are expressed as g dry weight for muscle. Statistical calculations were done according to Sædeco (37). The mean standard deviation (S.D.) for the TG concentration in one muscle biopsy calculated by analysis of variance from the 4-6 parts of the biopsy was 3.35 and 4.7 $\mu\text{mol/g}$ for controls, MI patients and HL subjects, corresponding to 10, 9.6 and 9.4% of the over all mean value for the TG concentration in the respective groups. The corresponding values for the cholesterol concentration in muscle was 0.17, 0.34 and 0.13 mg/g or given as percentage of the overall cholesterol concentration 11.1-8.5% in controls depending on age, 14.5% in MI patients and 8.0% in HL subjects.

RESULTS

Table II gives the mean values for the concentration of cholesterol and TG in plasma and muscle from control subjects and MI patients at different ages. As evident from the Table the cholesterol concentration in muscle from control subjects increased consistently from a mean of 1.53 mg/g in subjects aged 30-39 to 2.01 mg/g in the oldest age group. The TG concentration in muscle however showed no consistent changes with advancing age. No significant difference was found in the concentration of cholesterol or TG in plasma between the age groups.

The values for the concentration of lipid fractions in muscle from MI patients followed a pattern similar to that of control subjects. Thus the cholesterol concentration increased from the youngest to the oldest age group, although not as consistent as in control subjects, while the muscle TG concentration did not differ between age groups.

In Table III the values for muscle lipid fractions in control subjects and MI patients in the same age range (37-77 years) were compared. The mean cholesterol concentration in muscle was always higher in MI patients, significantly however only in 50-59-year-old subjects. The mean

Table II. Concentration of cholesterol in plasma (PL_{cho}) and in muscle (M_{cho}) and of TG in plasma (PL_{TG}) and in muscle (M_{TG}) from controls and MI patients (mean \pm S.E.M. range given within parentheses)

	Age (yr.)	PL_{cho} (mg/100 ml)	PL_{TG} (mmol/l)	M_{cho} (mg/g)	M_{TG} (mmol/g)
Controls					
5	35.6 \pm 1.0 (30-39)	206 \pm 20.9 (200-267)	1.46 \pm 0.18 (1.13-2.12)	1.53 \pm 0.09 (1.17-1.70)	29.6 \pm 4.0 (15.1-38.3)
7	44.0 \pm 1.3 (40-49)	238 \pm 8.6 (205-260)	1.49 \pm 0.09 (1.22-1.81)	1.71 \pm 0.05 (1.48-1.80)	26.7 \pm 2.2 (18.0-36.2)
25	51.0 \pm 0.5 (50-59)	228 \pm 7.0 (166-299)	1.50 \pm 0.06 (1.05-2.02)	1.77 \pm 0.06 (1.25-2.78)	30.4 \pm 1.9 (10.5-50.9)
10	65.5 \pm 1.4 (60-72)	234 \pm 10.2 (192-283)	1.52 \pm 0.06 (1.31-1.87)	2.01 \pm 0.09 (1.46-2.51)	37.5 \pm 2.8 (15.1-41.7)
47	51.4 \pm 1.4	228 \pm 5.0	1.50 \pm 0.04	1.79 \pm 0.04	29.1 \pm 1.3
MI patients					
6	44.3 \pm 1.7 (37-49)	281 \pm 17.6 (248-358)	3.0 \pm 0.32 (2.76-4.40)	1.85 \pm 0.15 (1.47-2.29)	31.8 \pm 5.3 (16.5-46.7)
18	54.4 \pm 0.7 (50-59)	274 \pm 9.6 (218-353)	2.89 \pm 0.32 (1.62-6.40)	2.36 \pm 0.12 (1.33-3.73)	37.5 \pm 4.4 (16.4-82.7)
16	62.9 \pm 0.6 (60-69)	275 \pm 12.7 (196-373)	2.35 \pm 0.20 (1.58-4.49)	2.39 \pm 0.18 (1.58-4.43)	37.5 \pm 4.6 (14.5-94.6)
10	75.9 \pm 1.1 (70-81)	49 \pm 13.1 (181-306)	2.08 \pm 0.16 (1.30-3.96)	2.74 \pm 0.34 (1.89-3.75)	36.1 \pm 3.4 (20.8-52.3)
50	66.2 \pm 1.5	270 \pm 6.3	2.63 \pm 0.14	2.35 \pm 0.10	36.5 \pm 2.3

cholesterol concentration, calculated as mean value for control subjects and MI patients, was higher in MI patients.

The concentration of cholesterol and TG in plasma was, however, higher in MI patients than in control subjects (Table II). Therefore, the data for MI patients were further analysed on the basis of the plasma lipoprotein pattern according to the criteria used clinically in this department, based upon the WHO recommendation (2) (Table IV). No significant difference was found for the concentration of cholesterol or TG in muscle between patients with normal, type IV or type IIB lipoprotein pattern. High values for the cholesterol concentration in muscle were found in two patients, one with a type IIA, the other

with a type III lipoprotein pattern both had high values also in the muscle TG concentration.

Table V shows that the concentration of TG in muscle was correlated to the concentration of TG in plasma in the controls, but not to age. On the other hand the concentration of cholesterol in muscle was correlated to age but not to the plasma cholesterol concentration. No correlation was, however, found between age and the concentration of TG in plasma, nor between age and the cholesterol concentration in plasma.

In contrast to the findings in control subjects, significant correlations could not be demonstrated either between age and the cholesterol concentration in muscle or between the TG concentration in plasma and muscle. As in control

Table III. Comparison of the concentration of cholesterol and TG between muscle from controls (C) and MI patients in the same age range (number of subjects within parentheses)

	Age group				
	37-49	50-59	60-72	37-72	37-42
	Cholesterol (mg/g)			Triglycerides (mmol/g)	
C	1.44 \pm 0.07 (9)	1.77 \pm 0.06 (25)	2.01 \pm 0.09 (10)	1.80 \pm 0.05	29.3 \pm 1.3
MI	1.85 \pm 0.15 (6)	2.36 \pm 0.12 (18)	2.50 \pm 0.27 (17)	2.34 \pm 0.13	37.0 \pm 2.7
P	> 0.05	< 0.001	> 0.05	< 0.001	< 0.01

Table IV Concentration of cholesterol in muscle (M_{cho}) and TG in plasma (PL_{TG}) and muscle (M_{TG}) from AII patients in relation to the type of lipoproteinemia (2)

N = normal lipoprotein pattern

	N	IV	IIIB	IIA	III
Age (yr)	65.2 ± 2.5	57.0 ± 2.0	60.7 ± 3.9	70	56
PL_{TG} (mmol/l)	1.79 ± 0.06	2.91 ± 0.17	3.06 ± 0.30	2.04	6.40
M_{cho} (mg/g)	2.3 ± 0.10	2.21 ± 0.12	2.35 ± 0.19	5.75	3.73
M_{TG} (mmol/g)	40.1 ± 4.9	32.8 ± 2.3	32.4 ± 6.5	52.3	82.7
n	16	23	7	1	1

subjects, no correlation was found between the concentration of cholesterol or TG in plasma and age.

In order to further investigate the relation between the concentration of TG in plasma and muscle, the following comparison was made. The control subjects were divided into four subgroups on the basis of plasma TG concentration, the subjects with HL into two subgroups (Table VI). The mean value for the TG concentration in muscle was then calculated for the subjects in each subgroup. It is quite clear from Table VI that values for the TG concentration in plasma above normal limits were not associated with elevated levels of TG concentration in muscle. The mean cholesterol concentration in muscle from the HL subjects was 1.6 ± 0.07 mg/g ($n = 1$).

DISCUSSION

In the present study a relationship was demonstrated between the cholesterol concentration in muscle and the age of healthy normolipemic men. This is in accordance with previous findings on autopsy material, where the cholesterol concentration in muscle was higher in older subjects regarded as a group in comparison to younger (14).

We have previously reported that the cholesterol concentration increased with age not only in muscle but also in plasma, in the rat (10). The cholesterol concentration in plasma increased, however at a considerably earlier time in the life span than in the muscle. Furthermore, physical training reduced the concentration of cholesterol in plasma but not in muscle (8). These findings indicate that no immediate relationship exists between the metabolism of cholesterol in plasma and muscle, a hypothesis which is in accord-

ance with the lack of correlation between the muscle cholesterol concentration and the total cholesterol concentration in plasma observed here and previously by Hood et al. (26).

An interesting feature in this context is the elevated level of cholesterol in erythrocytes in lecithin-cholesterol acyltransferase (LCAT) deficiency both in the familial form (23, 34) and in association with liver disease (22). The elevated level of cholesterol in red cells from patients with LCAT deficiency (34) could be reduced by incubation in plasma with normal LCAT activity. Norum et al. (35) suggested that esterification of free cholesterol in plasma by the LCAT reaction might be one means of preventing cholesterol accumulation in membranes.

Stein et al. (38) reported increased levels of cholesterol as well as of phospholipids in rat aorta with advancing age. They ascribed this increase to increased amounts of plasma membranes mainly in the smooth muscle cells. In skeletal muscle, however, an apparently age inherent increase in the cholesterol concentration may occur without change in the phospholipid concentration (10, 20). This may indicate either a shift in the proportion cholesterol/phospholipids in muscle membranes with advancing age or that cholesterol accumulates in non-membranous compartments. It may be of interest in this context that physical training increased the amount of cholesterol and phospholipids in rat muscle (21). This suggests increased amounts of membranous material in general in trained muscle; in patients suffering from arterial insufficiency an increased concentration of phospholipids was accompanied by increased activity of succinic oxidase activity (23), suggesting increased amounts of mitochondrial material in the affected muscle region, probably due to hypoxic adaptation.

Table V. Correlation coefficients for controls and MI patients

Age in years, cholesterol concentration mg/100 ml plasma (PL_{cho}) and mg/g muscle (M_{cho}), TG concentration mmol/l plasma (PL_{TG}) and μ mol/g muscle (M_{TG}).

<i>r</i>		<i>P</i>	
Controls (n = 47)			
Age	PL_{TG}	0.03	>0.05
Age	PL_{cho}	0.12	>0.05
Age	M_{TG}	-0.07	>0.05
Age	M_{cho}	0.43	<0.001
PL_{TG}	M_{TG}	0.30	<0.05
PL_{cho}	M_{cho}	0.04	>0.05
MI patients (n = 50)			
Age	M_{TG}	0.07	>0.05
Age	M_{cho}	0.23	>0.05
PL_{TG}	M_{TG}	0.04	>0.05
PL_{cho}	M_{cho}	0.13	>0.05

The apparent increase in the muscle cholesterol concentration from MI patients may indicate that these patients more readily accumulate cholesterol in muscle than healthy subjects. Kahn et al. (23) observed an increased concentration of cholesterol in muscle and in plasma from prairie dog and ground squirrel fed a hypercholesterolemic diet, and Bieberdorf and Wilson (4) reported results that do not exclude the possibility that a diet rich in unsaturated fatty acids lowered the plasma cholesterol concentration by causing a redistribution of cholesterol from plasma to muscle in rabbit. The lack of correlation between the concentration of cholesterol in muscle and plasma from the MI patients does, however, indicate that the elevated cholesterol level in muscle from these patients was not due merely to trapping of cholesterol from plasma.

An increased muscle cholesterol concentration may also be due to reduced removal of cholesterol from the muscle tissue. This possibility is of interest in view of a lowered level of HD lipoprotein in plasma of the MI patients (Carlsson and Eriksson, personal communication) since the HD lipoprotein is of importance for the LCAT reaction by forming a complex with the enzyme (35). In patients with a low LCAT activity a positive correlation was found between the concentration of cholesterol in erythrocytes and the concentration of free cholesterol in plasma (22). In the present study however the total plasma cholesterol concentration was determined.

The relationship previously reported between the concentration of TG in plasma and muscle (20) was confirmed. This relationship was, however weak and disappeared when also subjects with elevated levels of plasma TG were considered, e.g. an abnormal elevation of the plasma TG concentration is apparently not per se associated with elevated TG levels in muscle. This is also in line with findings in the VII patients, the highest values for the muscle TG concentration being found in those with a normal type of lipoprotein pattern.

An inverse relationship between the rate of elimination of TG from plasma and the level of plasma TG has previously been reported (5, 6). This may indicate that the flow of plasma TG fatty acids into, e.g., muscle is not necessarily increased with an elevated plasma TG concentration. It should be noted in this context that the plasma FFA is likely to be a more prominent determinant for the total fatty acid flux into muscle than the plasma TG and therefore also

Table VI. Concentration of TG in muscle (M_{TG}) from controls and from men with HL. Subgroups are constructed on the basis of the plasma TG concentration (PL_{TG})

Age 63		PL_{TG} (mmol/l)		M_{TG} (μ mol/g)	
$M \pm S.E.M.$	Range	$M \pm S.E.M.$	Range	$M \pm S.E.M.$	
Control subjects					
12	49.2 \pm 1.6	1.05-1.30	1.17 \pm 0.02	15.1	33.9
12	51.9 \pm 3.1	1.31-1.47	1.37 \pm 0.01	10.5-46.9	29.0 \pm 2.9
12	54.8 \pm 3.0	1.48-1.68	1.57 \pm 0.02	19.9-30.9	33.6 \pm 2.2
11	49.5 \pm 2.0	1.78-2.12	1.92 \pm 0.03	21.1-48.1	30.0 \pm 2.1
HL subjects					
10	30	2.10-2.52	2.28 \pm 0.05	18.0-42.1	27.1 \pm 2.7
11	30	2.60-3.99	3.63 \pm 1.01	18.6-42.5	30.1 \pm 2.4

for the rate of *de novo* synthesis of muscle TG. Thus in alloxan diabetic rat the increased concentration of TG in myocardium (36) and skeletal muscle (15) has been attributed to increased availability of plasma FFA, in turn partly due to loss of the insulin-suppressing effect on the adipose tissue lipolysis. Disturbances in the carbohydrate metabolism are frequent in MI patients (12). In three of the MI patients with untreated diabetes mellitus, not reported in the results, the muscle TG concentration was 52.0, 58.5 and 59.8 $\mu\text{mol/g}$.

ACKNOWLEDGEMENT

Supported by grants from the Swedish Nutritional Research Foundation and the Research Fund of the University of Uppsala.

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ELEVATED GLOMERULAR FILTRATION RATE IN INSULIN TREATED SHORT TERM DIABETES

Non-dependence on Blood Sugar Value

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Abstract. The glomerular filtration rate (GFR) has been measured in 34 male juvenile diabetics with a duration of diabetes of 1-12 years. The GFR was clearly elevated, as found earlier. There was no correlation to blood sugar levels. Even in patients with blood sugar values from 50 to 100 mg/100 ml the GFR was elevated to the same extent as in patients with higher values. The actual blood sugar during the clearance procedure cannot therefore be of importance for the high GFR observed in diabetic patients.

The glomerular filtration rate (GFR) is elevated in early juvenile diabetes (3-7, 10). It has been demonstrated that the high GFR is associated with the metabolic derangement in diabetes, since the GFR could be normalized during insulin treatment in newly diagnosed diabetes (4).

However in a limited series of insulin-treated patients published earlier (5), there was no demonstrable correlation between the GFR and the blood sugar measured at the time of the renal function test.

Experimental studies with glucose loading have led to contradictory conclusions (1, 2, 6).

We now present the results from simultaneous GFR and blood sugar determinations in a series of 34 patients, showing that there was no difference between GFR in patients with high or low blood sugar at the moment when the clearance study was carried out.

METHODS AND SUBJECTS

Thirty-eight male diabetic patients, aged 17-34 years, all with duration of diabetes of 1-12 years, were investigated. Pertinent data of age, body surface and duration of diabetes appear from Table I along with the results.

The GFR was determined using constant infusion technique as described in detail elsewhere (1). In 16 subjects I-125-iothalamate clearance and in 12 subjects insulin clearance were used for measuring GFR. Fifty-nine normal male subjects, aged 20-30 years, served as controls. All clearance values were corrected to 1.73 m² BSA.

All subjects were randomized in the fasting state. The diabetics did not receive their morning insulin until after the clearance test. No special effort had been made to ensure strict control of diabetes on the day before the test, but many of the patients turned out to have blood sugar between 50 and 150 mg/100 ml, as appears from the Table. Blood sugar levels were measured either by an ortho-toluidine method on capillary blood or by glucose oxidase method on serum. These samples were taken before the clearance tests. Most patients are examined ambulatory.

RESULTS

The results are shown in Table I. The diabetics are divided according to the actual blood sugar value into four groups: 50-100, 101-150, 151-200, and 201-300 mg/100 ml. The individual results appear from Fig. 1.

It is evident from the Table and the Figure that there is no correlation between the blood sugar level and the GFR. All GFR values are elevated to approximately the same extent in the four groups. The rank sum test according to Wilcoxon revealed that in all groups the level of statistical significance was less than 0.01.

DISCUSSION

The elevation of GFR in diabetes mellitus has been known for some years (10). Recently it has been demonstrated that this abnormality is re-

Table 1. Clinical data and results for the patients investigated

Blood sugar levels (mg/100 ml)	Clearance substances ^a	No. of subjects	Mean blood sugar (mg/100 ml)	Age (yr)	BSA (m ²)	Duration of diabetes (yr)	GFR (ml/min)
40-100	1	6	77.7 ± 10.3	22.3 ± 3.8	1.850 ± 0.079	5.7 ± 3.4	144.2 ± 8.9
	2	8	75.3 ± 13.3	22.8 ± 3.3	1.844 ± 0.069	7.3 ± 4.1	142.4 ± 9.0
101-150	1	5	126.8 ± 16.2	21.8 ± 5.5	1.876 ± 0.136	4.4 ± 2.4	141.8 ± 11.9
	2	10	177.4 ± 13.7	24.6 ± 5.4	1.891 ± 0.105	5.1 ± 3.6	142.0 ± 13.3
151-200	1	12	186.9 ± 27.5	23.9 ± 5.1	1.799 ± 0.144	4.8 ± 2.3	136.6 ± 17.4
	2	18	183.6 ± 76.7	4.9 ± 5.5	1.812 ± 0.139	5.1 ± 2.2	134.8 ± 16.8
201-300	1	3	221.0 ± 16.5	17.7 ± 0.5	1.620 ± 0.096	2.3 ± 1.5	139.0 ± 15.7
	2	6	148.7 ± 32.7	20.0 ± 3.9	1.693 ± 0.107	3.8 ± 3.1	151.8 ± 25.6
50-300	1	26	154.1 ± 54.9	22.4 ± 4.8	1.805 ± 0.140	4.6 ± 2.5	141.9 ± 15.6
	2	34	156.3 ± 61.5	23.6 ± 5.0	1.821 ± 0.127	5.3 ± 3.2	141.0 ± 16.7
Normal subjects	1	44		25.3 ± 2.5	1.910 ± 0.111		112.9 ± 13.8
	2	59		4.8 ± 2.6	1.905 ± 0.110		112.0 ± 13.3

1 = I-125-iothalamate clearance, 2 = I-125-iothalamate and inulin clearance.

sible: it disappears in the course of weeks when very precise blood sugar regulation is obtained (4).

One might have expected, therefore, that a correlation would be found if the blood sugar values measured at the time of the renal function test were plotted against the GFR value obtained. However in a series published earlier this was not the case (5). Also in long-term diabetics without proteinuria no correlation between GFR and blood sugar was found (8).

The present larger series confirms our earlier impressions. The average GFR is practically the same all the way from the group with blood sugar values of 40-100 up to that with blood sugar 201-300 at the moment when the renal function test was performed.

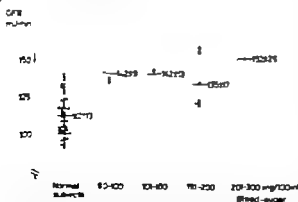


Fig. 1 GFR as measured by I-125-iothalamate clearance or inulin clearance in 59 young normal male subjects and in 38 young short-term male diabetics (diabetes duration < 12 years) with various blood sugar levels.

In an earlier study we tried to elucidate the problem experimentally. We gave glucose orally or intravenously to see whether induced hyperglycemia would result in an increase of the GFR. However the result was negative. Oral loading did not change the GFR, and i.v. glucose resulted in a small rise that corresponded to that expected with the slight change in plasma colloid osmotic pressure induced by the infusion (6, 7).

On the other hand, Bröchner-Mortensen (1, 7) reported an increase in GFR in normals during a combined oral and i.v. elevation of blood sugar and suggested that the elevation of blood sugar per se partly explained the small increase in GFR observed. He also suggested that the elevated blood sugar found in diabetics could explain at least in part the high GFR found in these patients.

Our present results do not support this concept. Elevated GFR is found in patients with normal or low blood sugar levels (50-100 mg/100 ml) and also in patients with slightly elevated blood sugar levels, 100-150 mg/100 ml, concentrations which do not produce an increase in GFR in normals (6). Therefore high blood sugar during clearance tests in diabetics is not the explanation of the high GFR in these patients.

It must in some way be due to the metabolic aberrations present in diabetic patients being treated in the usual way with diet and insulin. In daily practice it is very seldom possible to obtain a normal diurnal blood sugar level, day by day and year by year.

During hospitalization, when the diet is strictly

controlled and insulin is given according to the results of multiple blood sugar determinations during the day complete or nearly complete normalization of the metabolic situation can, however be maintained for days or weeks. In this artificial situation the GFR falls to normal levels (4).

The mechanism behind the high GFR in diabetes is not clear. Increased kidney size has, however been found in 12 patients with early juvenile diabetes. As measured on X-rays the kidney size was elevated in approximately the same extent as the GFR (9). This finding suggests that the mechanism behind the high GFR in diabetes may be elevated kidney size and glomerular size, which may possibly be mediated through elevated growth hormone secretion (7).

ACKNOWLEDGEMENT

The investigations are supported by grants from Svenska Insulin diabetes Forskningsråd.

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CHRONIC CONGESTIVE HEART FAILURE TREATED WITH LONG TERM INFUSION OF GLUCAGON

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Abstract. Eight patients with severe chronic congestive heart failure (functional classes III-IV N.Y.H.A.) have been treated with continuous infusion of glucagon (2-4 mg/h) for 96 hours. During this period all other treatment was kept constant. Mean b.wt. was reduced from 62.8 kg to 61.0 kg ($p < 0.02$) and there was a slight decrease in the serum concentrations and urinary excretion of sodium, potassium and chloride. Four patients showed a slight improvement in dyspnoea, otherwise no beneficial clinical effects were found. All patients completed the treatment. The possible influence of the metabolic changes induced by long-term glucagon infusion on chronic congestive heart failure is discussed.

The clinical effect of glucagon therapy is well documented in acute heart failure and after open heart surgery (2, 4, 5, 6, 9, 11). In chronic congestive heart failure, however, the effect is more controversial (7).

We have studied the cardiac and metabolic effects of glucagon given intravenously by continuous infusion for 96 hours in eight patients with serious chronic congestive heart failure. The results of the metabolic studies are published elsewhere (1).

MATERIAL AND METHODS

Three females and five males were studied. Their mean age was 65 years (median 63). Table I gives clinical information concerning diagnosis, classification of the cardiac function according to the New York Heart Association, and dosage of glucagon. The patients were on constant-dosage cardiac insufficiency regime (digitalis, diuretics, low salt diet) at least one week before and during the whole period of treatment with glucagon. In addition, morphine was given in doses of 4 mg b.i.d. as added from 2 days before infusion started in order to reduce nausea. Glucagon, supplied by Novo Industry, was given in 2.5% fructose solution (30 ml/h) from a constant rate infusion pump. The rate of infusion of glucagon was 2 mg/h in four patients and 4 mg/h in four

The plan of the study is illustrated in Table I. Determinations of b.wt., BP, pulse rate, serum sodium, potassium and chloride, in serum, the excretion of these electrolytes, blood pH and pCO_2 were performed. ECG was recorded daily. Just after the treatment period chest X-ray, arm-to-tongue circulation time as determined by injection of sodium dihydrobosphate (Dechobol).

Sodium and potassium were measured by flame spectrometry in serum on a Beckman automatic flame photometer (Aa-Ekbohm International standard) and in urine on an Eel flame photometer. Chlorides in serum and urine were measured by the coulometric titration method on an Eel chloridometer. Blood pH and standard bicarbonate in arterialised capillary blood were measured on Astrup equipment, Radiometer.

RESULTS

There was a slight improvement in the feeling of dyspnoea in patients 1, 2, 3 and 4. Circulation time was measured in six of the patients and was reduced in five of them (Fig. 1). The mean value of circulation time, however, increased from 32.5 sec to 33 sec, due to patient 7. Fig. 2 illustrates the changes in b.wt. Mean weight was reduced from 62.8 to 61.0 kg, a statistically significant reduction ($p < 0.02$).

Table III shows the values for serum electrolytes, the urinary excretion of the electrolytes, blood pH and standard bicarbonate before and during glucagon treatment. There was a slight decrease in the serum concentration and urinary excretion of the electrolytes, but the difference was significant only for the urinary excretion of chlorides ($p < 0.02$). Blood pH and standard bicarbonate were unchanged. A change in pulse rate, BP, chest X-ray or ECG was not observed. Nausea during most of the treatment period was observed in all patients. The volume limited by shown in Table IV.

Table I. Clinical features of the patients studied and the glucagon dosage

Group I clinical effect, group II no clinical effect

CHD = coronary heart disease, PMD = primary myocardial disease, MI = mitral insufficiency, AS = aortic stenosis, PE/T = pulmonary embolism/thrombosis, op. = operated

Group	Case no.	Sex	Age (yr)	Diagnosis	Functional class (NYHA.)	Heart rhythm	X-ray heart ol. (ml/m ²)	Glucagon dosage (mg/kg)
I	1	♀	77	CHD MI	IV	Sinus	990	4
	2	♀	62	PMD	III	Atr fibr	1 200	2
	3	♂	48	MI op.	IV	Atr fibr	830	4
	4	♂	52	CHD MI	III IV	Sinus	760	2
II	5	♂	66	CHD	IV	Sinus	1 400	2
	6	♂	61	CHD MI PE/T	IV	Sinus	930	4
	7	♂	62	CHD AS MI	III IV	Sinus	625	2
	8	♂	88	AS	IV	Sinus	670	4

DISCUSSION

Several reports have stated a positive inotropic effect of glucagon given intravenously during heart catheterization (3, 10, 11). The increased cardiac output and ventricular stroke volume has been reported to be similar to that of digitalis and to persist during blockade. It is possible that glucagon is the drug of choice in heart failure when propranolol (7).

In the present study, heart failure, only a few cases without any change in heart failure without any other therapy. The result was not significant and the clinical effect in heart failure was not improved in eight patients.

Recently Lvoff and Wilken (4) treated 12 patients with chronic congestive heart failure unresponsive to all other therapies. Eight of the patients improved during glucagon therapy in three of them died in the hospital.

The evaluation of the effect of the present therapeutic trial is difficult. The reduction in b.w.t. might be the result of either improvement of heart failure or lowered food intake because of nausea or both. As shown in Table IV, there seemed to be a connection between the reduction

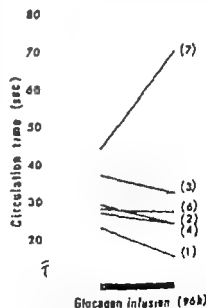


Fig. 1 Circulation time arm-to-tongue determined by sodium dihydrobolyte (Decholler) before and after glucagon infusion. Figures within parentheses indicate patient no. — Individual values, --- mean values.

B.w.t.
BP
Pulse rate
ECG
N } in urine/24
K } serum conc
Cl }
Blood pH, stand. by
Circulation time
Chest X-ray

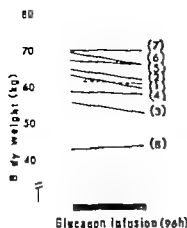


Fig. 2. Body weight before and after glucagon infusion. Symbols as in Fig. 1.

of b.wt. and the volume omitted. A few determinations of circulation time do not constitute a good test for small fluctuations in chronic congestive heart failure. Increased urinary excretion of electrolytes usually occurs during improvement of heart failure. In our cases there was a significant reduction of urinary chloride excretion, but that might be partly explained by loss from gastric fluid. Serum sodium decreased in spite of a slight decrease of urinary sodium excretion. These changes might be a result of dilution, but we suggest that the intake of electrolytes was reduced during the therapeutic period, due to nausea. The fructose infusion (30 ml/h) may also be of importance in respect of lowered serum sodium.

The patients were all severely ill and com-

plained of dyspnoea. The clinical impression, mainly changes in dyspnoea, must be of great importance in evaluation of the therapy. A slight improvement of dyspnoea was noticed in four of our patients (Table I). We feel, however that this improvement was overshadowed by the unpleasant nausea.

The metabolic studies (1) revealed that glucagon induced a state of mild carbohydrate intolerance, characterized by slight increase in fasting blood sugar and decrease in glucose disappearance rate in spite of considerable rises in serum insulin. This diabetes-like state may be accounted for by the elevated levels of serum growth hormone. The failing hypoxic heart derives the major part of its energy requirements from the metabolism of glucose (8). As insulin is essential for the normal uptake of glucose by the myocardium, the insulin resistance induced by glucagon treatment may thus have negative implications in these patients with severe heart failure. These effects may partly explain the negative results of long-term glucagon treatment in contrast to the marked inotropic effect of single injections.

The conclusion of this study is that long-term infusion of pharmacological doses of glucagon to patients with severe chronic congestive heart failure may have a slight effect in some patients, but the treatment is very unpleasant and induces considerable metabolic effects, which might even worsen the heart failure. We feel that glucagon infusions have no place in the routine management of these patients.

Table III. Blood pH and standard bicarbonate serum concentration and urinary excretion of sodium, potassium and chlorides before and during glucagon infusion.

Pat. no.	Before glucagon (mean of 2 days)							During glucagon infusion (mean of 2nd-4th day)						
	Serum (mEq/l)			Urine (mEq/24 h)				Serum (mEq/l)			Urine (mEq/24 h)			
	Na	K	Cl	Na	K	Cl	pH	Na	K	Cl	Na	K	Cl	pH
1	132	5.2	97	45	50	102	7.48	136	4.0	98	44	51	56	7.42
2	142	4.6	117				7.43	140	4.1	91				7.30
3	137	4.5	94	66	69	107	7.46	133	4.6	91	38	60	71	7.45
4	141	4.1	107	33	62	90	7.51	134	4.0	103	53	44	36	7.47
5	136	3.8	95	56	84	97	7.42	130	3.6	95	20	66	31	7.45
6	138	4.2	92	31	75	32	7.45	135	3.5	92	69	38	35	7.46
7	140	4.0	103	5	62	19	7.41	138	4.5	103	10	39	3	7.45
8	140	3.9	101	43	26	28	7.45	133	3.9	92	8	78	10	7.46
Mean	138.2	4.78	100.8	45.5	61.1	64.1	7.45	135.1	4.02	96.3	34.5	52.2	34.5	7.45

Table IV Relation between glucagon dosage volume vomited and weight change

Case no.	Glucagon (mg/kg)	Vomited (1/96 h)	Weight change (kg/96 h)
1	4	2.1	-3.6
2	2	2.5	-3.0
3	4	0.7	-4.0
4	2	0	-1.2
5	3	0	-1.5
6	4	0.4	-3.3
7	3	0	-0.3
8	4	0.8	+0.6

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THE EFFECT OF DIFFERENT SERUM CONCENTRATIONS OF ANTIMICROBIAL DRUGS ON THE LACTOBACILLUS LEICHMANNII VITAMIN B₁₂ ASSAY

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Abstract. The existence of antimicrobial drugs in serum may invalidate microbiological serum assays by inhibiting the growth of the test organism. Sixteen antimicrobial agents have been added to serum *in vitro* and tested for their ability to influence the vitamin B₁₂ assay by the *L. leichmannii* method. Seven were found to cause significant inhibition when screened at very high serum levels. On further examination the lowest serum concentrations needed to reduce the estimated vitamin B₁₂ by as much as 25% were 1.3 µg/ml phenoxymethylpenicillin, just below 2.5 µg/ml Bacitracin, 3-10 µg/ml nafcillin, 10-25 µg/ml benzylpenicillin, carbenicillin or erythromycin, and 25-125 µg/ml chloramphenicol.

It is generally accepted that the microbiological assay of vitamin B₁₂ may be invalidated if serum contains antimicrobial drugs (9). This has been investigated by several authors using *L. leichmannii* as test organism (2, 6, 8, 10). The results however are conflicting. The amount of drug present in serum is an important factor and the purpose of the present investigation is to report how the vitamin B₁₂ assay with *L. leichmannii* was affected by different serum concentrations of antimicrobial drugs *in vitro*.

METHODS

Two drug powders were dissolved in distilled water. Serial dilutions were made and from these 0.2 ml was added to 3.8 ml of fresh human serum. The vitamin B₁₂ assay of this slightly diluted serum was then started within one hour.

Serum vitamin B₁₂ determinations were performed at the laboratory of Krokgården Hospital. The procedure is based on the method described by Mandelstam and Weikla (5) using *L. leichmannii* (ATCC 7830) and Bacto B₁₂ assay medium USP. Some minor modifications are employed.

For extraction as aqueous solution is used which con-

tains 1.2 g anhydrous citric acid, 0.1 g sodium metabisulphite and 1.62 g disodium phosphate per 100 ml. The mixture of serum and extraction solution is autoclaved at 118°C for 5 min and duplicate 0.5 ml portions of the supernatant are transferred to the assay tubes. The final dilution of serum is 1/60.

The Bacto B₁₂ assay medium is prepared by dissolving 50 g in 750 ml distilled sterile water. Before boiling for 2 minutes 6 drops of Tween 80 are added. The assay tubes are autoclaved at 118°C for 5 min prior to inoculation.

A 20-hour growth of *L. leichmannii* is centrifuged and washed 3 times in sterile solution containing 5 ml medium and 5 ml distilled autoclaved water. The precipitate is suspended in another 10 ml, and 1-2 ml (depending on the turbidity) is transferred to 10 ml of the water-medium solution and placed in an agitator for 5 min. The samples are inoculated with 2 drops of this solution. The tubes are incubated at 37°C until the most concentrated standard solution gives reading of 0.5 on Linco 3 Photometer (Ljungberg & Co., Sweden), usually 20-22 hours. The tubes are properly chilled before reading, which is performed automatically by an Astrolab Dataconverter 601 (Ljungberg & Co., Sweden).

RESULTS

Sixteen antimicrobial drugs were screened for inhibition at a very high serum concentration *in vitro*. For 14 drugs 250 µg/ml was chosen, whereas chloramphenicol was tested at 125 µg/ml for technical reasons because of its low solubility in water. Trimethoprim was tested at 10 µg/ml. This far exceeds what can be expected after conventional doses when used in combination with sulphamethoxazole (7). The results are given in Table I.

Cephalothin and tetracycline which had caused moderate depression of growth, were further examined twice at the concentrations of 50 and

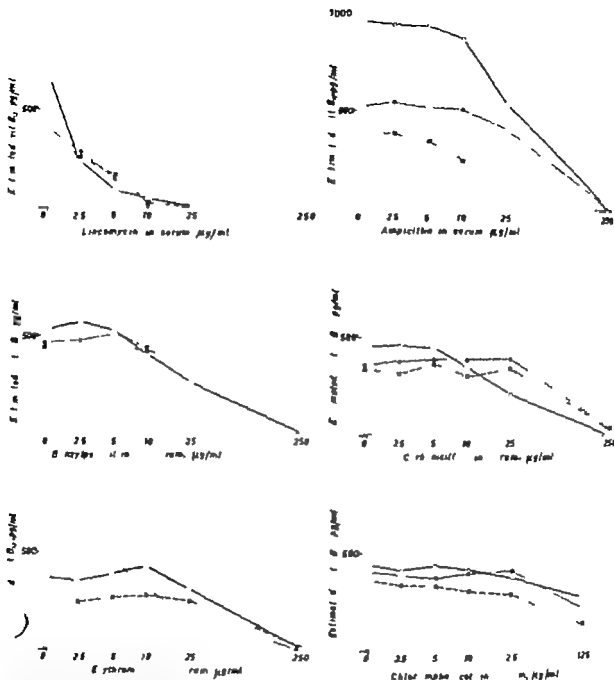


Fig. 1. The effect of increasing serum concentrations of six antibiotics on the vitamin B₁₂ assay. Each agent was tested three times.

250 µg/ml. However not even 250 µg/ml reduced the estimated vitamin B₁₂ by as much as 15% in any of the assays. This indicates that for these two drugs 250 µg/ml is near the lower limit of significant inhibition.

The seven drugs that had caused severe inhibition were examined more extensively. Except for

phenoxymethylpenicillin, serum concentrations of 250, 25, 10, 5, 2.5 and 0 µg/ml were prepared. The assays were performed with each antibiotic on 3 different days and as a rule with serum from different persons. The results are given in Fig. 1. Phenoxymethylpenicillin was tested in smaller concentrations. The inhibition curves are shown

Table 1. Result of screening antimicrobial drugs at a serum concentration of 250 µg/ml for inhibiting the growth of *L. leichmannii* in the vitamin B₁₂ assay

Very inhibition (Reduction of optical density > 50%)	Moderate inhibition (Reduction of optical density 15-50%)	No inhibition (Reduction of optical density < 15%)
Phenoxyethylpenicillin Benzylpenicillin Ampicillin Carbenicillin Hydrocortisone Lincomycin Chloramphenicol ^a	Cephalothin Tetracycline	Methicillin Oxytetracycline Streptomycin Sulphamethizole Sulphamethoxazole Sulphamethoxypyridazine Trimethoprim ^b
Screened at serum concentration of 125 µg/ml		
Screened at serum concentration of 11 µg/ml		

in Fig. 2. As could be expected, all the drugs did not affect the assay to the same degree. There are also differences in the amount of inhibition caused by the same antibiotic in the 3 assays.

The lowest serum level that can cause inhibition is the one of practical importance. The concentrations needed to reduce the estimated vitamin B₁₂ by as much as 25% in at least 1 out of the 3 assays were 1-2 µg/ml phenoxyethylpenicillin, just below 2.5 µg/ml lincomycin, 5-10 µg/ml ampicillin, 10-15 µg/ml benzylpenicillin, carbenicillin or erythromycin, and 25-125 µg/ml chloramphenicol.

Common to all the inhibition curves was that reduction of growth took place gradually with increasing drug concentration. This means that inhibition, when occurring in the routine vitamin B₁₂ assay may be difficult to recognize.

DISCUSSION

Of the 16 antimicrobial drugs tested, 7 have been found able to inhibit the growth of *L. leichmannii* in the assay of vitamin B₁₂. Except for phenoxyethylpenicillin and lincomycin fairly high serum levels were needed. Nevertheless most of these levels may be exceeded in vivo.

According to Weinstein (11) the following relationship exists between dose and serum concentration. If 300 000 U (180 mg) benzylpenicillin is given intramuscularly a peak serum level of 8 µg/ml is reached. Similarly 10 g sodium ampicillin yields about 10 µg/ml, and 10 g carbenicillin 10-20 µg/ml. When 0.5 g lincomycin is given orally a peak serum level of 2-5 µg/ml is

obtained. Chloramphenicol reaches a serum level of 20-40 µg/ml when 2 g is given orally. As for erythromycin, a single oral dose of 0.5 g as the stearate gives a peak serum concentration of 2-20 µg/ml, and continued medication every 6 hours may yield peaks as high as 50 µg/ml. When phenoxyethylpenicillin is concerned, there is also a considerable variation between subjects. A single oral dose of 250 mg may after 1 hour give values ranging from <1 to about 6 µg/ml (3).

It was observed that each drug did not always cause the same degree of inhibition. This may partly be explained by the fact that the number of viable organisms added to the assay tubes may vary from day to day. Since autoclaving is known to reduce the activity of at least benzylpenicillin and ampicillin (1), minor inconsistencies in this procedure may also influence the result for these drugs. Inconstancy of inhibition was also reported

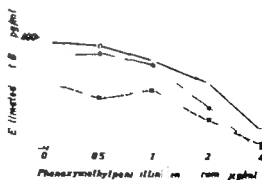


Fig. 2. The effect of increasing serum concentrations of phenoxyethylpenicillin on the vitamin B₁₂ assay tested three times in different sera.

by Powell et al. (6) for ampicillin. The significant difference between inhibition caused by phenoxymethylpenicillin and benzylpenicillin is probably due to more severe inactivation of the latter during extraction and heating.

Often it is not possible to perform the vitamin B₁₂ assay until a couple of days after the blood is collected. Penicillin does not significantly lose activity when serum is stored for 7 days at 4°C (4). In an aqueous solution the stability of the other antibiotics also appears to be good (10). Therefore it is not probable that the delay would seriously influence the degree of inhibition.

Concerning the 7 antibiotics, most of them are rapidly eliminated from serum in vivo. To avoid inhibition blood should therefore be taken in the morning before medication. The biological half life of lincomycin is as long as 5-6 hours (11). When a patient is receiving this drug, an alternative method for vitamin B₁₂ assay should be preferred.

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IMMUNOGLOBULINS IN PERNICIOUS ANAEMIA

Including a Report on a Patient with Pernicious Anaemia, IgA Deficiency and an M Component of Kappa-type IgG

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Abstract. Determinations have been made of the concentrations of serum total protein, electrophoretic fractions and immunoglobulins A, G and M in 11 patients with pernicious anaemia (PA) before and after treatment with vitamin B₁₂ and in 22 other patients with PA who had already received treatment. During the course of treatment a tendency was observed to increasing values of the serum globulin fractions and IgG. All the sera were subjected to immune electrophoresis. One patient with slight IgA deficiency exhibited pre-symptomatic myeloma (monoclonal gammopathy) of the IgG, kappa-type.

Autoimmune diseases, lymphoproliferative disorders and immunological deficiency syndromes are related in a number of ways. Patients with pernicious anaemia (PA) (5, 24) and autoimmune haemolytic anaemia (2) may have hypogammaglobulinaemia. Malignant lymphomas occur in association with Sjögren's syndrome (23), systemic lupus erythematosus and rheumatoid arthritis (3, 10), systemic scleroderma, dermatomyositis and polyarteritis nodosa (17). A combination of multiple myeloma and rheumatoid arthritis (26), as well as of pernicious anaemia and Sjögren's syndrome (25), in the same patients has also been found. On the other hand, patients with hypogammaglobulinaemia may develop autoimmune haemolytic anaemia (19), rheumatoid disease (20) and lymphomas or leukaemia (15). Finally patients with lymphoproliferative disorders, including chronic lymphatic leukaemia, frequently develop autoimmune haemolytic anaemia (22).

The combination of PA and myeloma is rare. In 1956 Mandema (12) described myeloma in a patient with known PA. Larsson (9) has reported

six cases of myeloma and three of benign monoclonal hyperglobulinaemia with the clinical picture of PA. Bichel (1) has drawn attention to PA and myxoedema in a patient with long-standing myeloma. In 1970 Gimsberg and Mullinar (7) described a patient with IgA deficiency who developed both PA and a monoclonal gammopathy.

In 1970 a patient with PA and a serum M component of IgG type was treated in our hospital. This observation led to determinations of the M components and immunoglobulins in patients with PA, treated and untreated. A report is given on the case and the results of the serum protein investigations.

MATERIAL AND METHODS

Thirty-three patients with PA, 22 women and 11 men, have been studied. The diagnosis of PA was established in all cases by finding macrocytic anaemia, typical blood picture, megaloblastic bone marrow spectrum, gastric achlorhydria, low concentration of serum vitamin B₁₂, pathological Schilling test without intrinsic factor, and prompt clinical response to parenteral treatment with vitamin B₁₂. Patients who did not fulfil these criteria were excluded, as also patients having other consistent diseases that might have influenced the concentration of serum proteins.

In 19 of 28 cases examined antibodies to gastric parietal cells were demonstrated, and in 7 of 10 cases the serum was found to contain blocking antibodies to the intrinsic factor.

Twenty-two patients (group B) had been treated with vitamin B₁₂ before the present study and were in good clinical remission at the time of examination. In 11 other cases (group A) the concentrations of the serum proteins were determined before and after treatment. The

Table I. Concentrations of serum total protein and electrophoretic fractions (g/100 ml) in groups A and B and healthy controls (mean \pm S.E.M. and S.D.)

	N	Total protein	Albumin	Globulins			
				α_1	α_2	β	γ
Group A							
Untreated	11	6.8 \pm 0.22 0.73	4.18 \pm 0.14 0.48	0.28 \pm 0.02 0.05	0.44 \pm 0.03 0.06	0.69 \pm 0.02 0.07	1.17 \pm 0.08 0.28
Treated	11	7.0 \pm 0.08 0.28	4.11 \pm 0.06 0.21	0.30 \pm 0.01 0.04	0.51 \pm 0.02 0.07	0.78 \pm 0.02 0.08	1.30 \pm 0.03 0.10
Group B	22	7.1 \pm 0.12 0.68	4.33 \pm 0.08 0.44	0.30 \pm 0.01 0.06	0.52 \pm 0.02 0.11	0.75 \pm 0.02 0.14	1.13 \pm 0.02 0.30
Controls	40	7.1 \pm 0.06 0.38	4.10 \pm 0.07 0.44	0.36 \pm 0.01 0.06	0.53 \pm 0.01 0.09	0.80 \pm 0.02 0.13	1.34 \pm 0.03 0.16

latter determinations were made 3-6 months after the treatment had begun.

Determination of the serum total protein concentration was made by the application of Reinhold's modification of Weichselbaum's method (18). Electrophoretic separation of the serum protein fractions was effected on cellulose acetate sheets in barbiturate buffer pH 8.6. A Beckman chromocam set was employed for densitometric calculation of the fractions (6). The immunoelectrophoretic micro-method of Scheidegger (21) was applied for the detection of serum M-components, with horse human antiserum and specific immunoglobulin antiserum. Quantitative determination of the serum immunoglobulins was effected by the radial immunodiffusion technique of Mancini et al. (11).

RESULTS

Serum total protein and electrophoretic fractions

The values for serum total protein and the different electrophoretic fractions are indicated in Table I.

In group A all the mean values of untreated fall within the normal ranges. The differences between these values and those of healthy

controls are not statistically significant. After treatment an increase in all globulin fractions is observable although the differences are not statistically significant.

In group B the levels of albumin and globulins correspond to those of the treated patients in group A. The differences between these values and those of the untreated patients in group A and of healthy controls are not statistically significant.

Serum immunoglobulins

The concentrations of serum immunoglobulins IgG, IgA and IgM are indicated in Table II. The mean values of all immunoglobulins in both groups are within the normal ranges.

In one untreated patient of group A an IgG value below the normal level (7.80 g/l) and two values above this (19.44 and 20.00 g/l) were noted. After treatment with vitamin B₁₂ all the values in group A were within the normal range; the mean value of IgG had increased but the difference was not statistically significant. In group B an IgG value of 31.30 g/l was noted, but no values below the normal level were observable.

In group A the concentration of IgA was normal in both untreated and treated patients. Four patients in group B were found to have IgA levels below the normal. In one of them no IgA was detectable. One patient had an IgA level above the normal upper limit.

An IgM value below the normal range was noted in one patient in group B. Only normal values were registered in group A.

Table II. Concentrations of immunoglobulins (g/l) in groups A and B (mean \pm S.E.M. and S.D.)

	N	IgG	IgA	IgM
Group A				
Untreated	11	13.82 \pm 1.19 3.96	3.56 \pm 0.45 1.30	0.81 \pm 0.18 0.58
Treated	11	14.32 \pm 0.53 1.78	3.18 \pm 0.21 0.68	0.88 \pm 0.11 0.35
Group B	22	14.68 \pm 0.73 4.19	3.14 \pm 0.26 1.48	0.87 \pm 0.10 0.59
Normal range		8.0-19.0	1.3-5.2	0.3-1.4

Serum protein M components

Immunoelectrophoresis of the serum proteins was carried out in all 33 patients. An M component was detected in only one patient, whose case history will be briefly presented.

CASE HISTORY

The patient, 78-year-old man, had been in good health until 1970, when he was admitted to the hospital on account of fatigue and increasing dyspnoea. A macrocytic anaemia as found, Hb 6.7 g/100 ml, RBC 4.52 mfl./mm³, reticulocytes 0.8%, MCH 44 pg, MCV 133 μ , WBC was 2 200/mm³ with normal differential count, but 10% hypersegmented neutrophils. The platelets were 89 000/mm³. The bone marrow specimen revealed megaloblastic erythropoiesis. Serum iron was 269 mg/100 ml, the iron-binding capacity 288 mg/100 ml. Free haaptoglobin was not detected, serum bilirubin was 1.7 mg/100 ml. The direct Coombs' test was negative. Serum vitamin B₁₂ concentration was low (50 pg/ml), folic acid concentration normal. Histamine refractory gastric achylia was demonstrated. The Schilling test without intrinsic factor showed an excretion of 1.1%, and with intrinsic factor 15.5%. Antibodies to parietal cell cytoplasm were detected in serum by the immunofluorescent technique (titre 1 100). Intrinsic factor blocking antibodies were not demonstrated.

These findings gave confirmation of the diagnosis of PA. The patient was treated with vitamin B₁₂ and rapid improvement occurred. A rise in the reticulocytes to 9.8% was noted.

After two weeks of treatment the megaloblasts in the bone marrow had disappeared and the erythropoiesis constituted 40% of all nucleated marrow cells. A new observation, not made in the first marrow specimen, was a markedly increased amount of plasma cells, 10% of the total number of marrow cells, and additionally groups of 5-10 cells. This finding led to further study of the serum proteins. The total serum protein concentration was 7.1 g/100 ml. The electrophoretically estimated protein fractions were: albumin 4.90, α_1 -globulin 0.30, α_2 -globulin 0.41, β_1 -globulin 0.41, β_2 -globulin 0.12, and γ -globulin 1.90 g/100 ml. The γ -globulin fraction was narrow and high. Immunoelectrophoresis showed normal albumin, haaptoglobin and IgM. The IgA line was hardly detectable. The IgG band displayed pathological fraction located in the middle of the band. Determinations with anti-IgG, anti-kappa and anti-lambda immunoserum showed that the M component was of an IgG, kappa-type. Quantitative determinations of the immunoglobulins gave the following results: IgG 15.00 g/l, IgA 1.90 and IgM 0.80 g/l. No proteinuria was present, and the test for Bence Jones protein was negative. Skull X-rays and bone survey did not provide any evidence of multiple myeloma. X-ray examinations of the kidneys, upper gastrointestinal tract and large bowel showed normal findings.

The patient has been symptom-free for more than 2 years. Repeated radiological examinations of the skeletal

system have not indicated signs of multiple myeloma. The bone marrow specimens contain 5-10% plasma cells. The serum protein fractions and immunoelectrophoresis have remained unchanged. One year subsequent to the diagnosis of PA the IgG concentration had risen to 19.00 g/l, the IgA was still low (1.25 g/l) and the IgM level unchanged (0.75 g/l).

DISCUSSION

Earlier reports have been made of the association of PA with decreased serum globulin values (13-24). A diminished level of γ -globulin in untreated patients with PA and normalization during treatment with vitamin B₁₂ have been reported by van Dommelen et al. (5). This tendency to increasing serum globulin levels during therapy has also been observable in the present study although the rise is not statistically significant.

Quantitative determinations of serum immunoglobulins in untreated patients with PA have given divergent results. Both reduced (4-24) and normal values (4-14) have been reported. These discrepancies are not necessarily in conflict, since Odgers and Wangel (14), in 15 of 20 patients with PA, found a diminution in the IgA-containing mononuclear cells of the gastric mucosa, despite normal serum IgA values. The theory has been put forward that deficiency of immunoglobulin may be a factor that increases the tendency to development of PA (9-24).

Of the 10 patients with diminished immunoglobulin levels described by Twomey et al. (24) none had antibodies to parietal cells of the gastric mucosa or to the intrinsic factor. The patient with IgA deficiency reported by Ginsberg and Mullhaax (7) had antibodies to the intrinsic factor. In the present series the only patient without detectable IgA had antibodies to parietal cells. Consequently IgA deficiency alone obviously does not reduce the capacity to produce antibodies to parietal cells or to the intrinsic factor. However of the nine patients in the present study without demonstrable antibodies to parietal cells one had a low concentration of IgG and another of IgM but all the patients had normal IgA levels. It seems possible that patients with general hypogammaglobulinaemia, those with solitary IgA deficiency and those with normal immunoglobulins, may represent distinct and perhaps genetically different types of PA.

The combination of PA and monoclonal gammopathy in one and the same patient is a rare finding. In animal models lymphoid malignancy or monoclonal gammopathy may develop after viral infections. In NZB mice an autoimmune disease apparently caused by a virus, develops, characterized by autoimmune haemolytic anaemia and glomerular nephritis. Lymphoid malignancies occur in about 25% of these mice (8). In Aleutian mink an apparent viral illness develops with renal lesions resembling those of lupus nephritis. In many of these minks a monoclonal gammopathy develops ultimately (16). A corresponding mechanism might possibly be of importance in patients with PA developing monoclonal gammopathy. On the other hand it is known that monoclonal gammopathy may occur secondarily to prolonged antigenic stimulation caused by tumours, chronic infections or autoimmunization.

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AGRANULOCYTOSIS CAUSED BY NITROFURANTOIN

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Abstract. Two episodes of agranulocytosis in connection with nitrofurantoin medication in one and the same female case are reported. In both instances rapid recovery followed treatment with prednisone.

Nitrofurantoin is one of the drugs in most common use when treating chronic pyelonephritis. It usually been regarded as haematologically safe, and is commonly used for periods of several months. During treatment with nitrofurantoin leucopenia has been reported in one patient with systemic lupus erythematosus (2) and in another with untreated polycythaemia (1). This report concerns a case of recurrent agranulocytosis after medication with nitrofurantoin.

CASE REPORT

A 62-year-old woman had suffered from urinary incontinence, and her uridine prolapse was operated upon in Oct. 20th, 1949. On the day of operation nitrofurantoin treatment at dosage of 150 mg daily was begun for urinary tract infection. There were no operative complications, and nitrofurantoin was continued ambulatorily. On Nov 16th she developed a cough and high fever. Her condition did not improve with oral phenoxymethylpenicillin during the subsequent three days. A blood count revealed leucopenia and she was referred to hospital on Nov 19th.

On admission she had fever of 39.3°C, her tonsils were enlarged and coated, rales could be heard on both lungs. Her blood count was Hb 141 g/l, leucocytes 0.6 $\cdot 10^9/l$ with 0.5% segmented neutrophils, 51.5% monocytes and 48% lymphocytes, platelets $21 \cdot 10^9/l$. A very small specimen from the bone marrow was obtained without any specific diagnostic characteristics. She was treated with β -penicillin, and from Nov 11th with oral prednisone, 60 mg daily. Her condition improved and on Nov 24th her WBC count was $2.9 \cdot 10^9/l$ with 1% band forms and 51% segmented neutrophils. She later made complete recovery both clinically and haematologically.

In Oct. 1971 she again developed a urinary tract infection and was treated with nitrofurantoin, 150 mg daily. In early November she developed fever and sore throat. She was given phenoxymethylpenicillin, and nitrofurantoin treatment was discontinued. Because of the continuing high fever she was admitted to hospital on Nov 10th. She had persistent tonsillitis and fever (40°C). Her blood count was: Hb 115 leucocytes 1.8 with 0.5% band neutrophils, 0.5% basophils, 20% monocytes and 79% lymphocytes. She was treated with penicillin and prednisolone, and three days later she was afebrile, her leucocytes were 3.6 with 1% myelocytes, 0.5% metamyelocytes, 3% band and 58% segmented neutrophils, 7% monocytes and 30.5% lymphocytes. She recovered completely.

DISCUSSION AND CONCLUSION

In our patient the role of nitrofurantoin as the causative agent of agranulocytosis is obvious; she had two episodes of agranulocytosis, in both instances in connection with a course of nitrofurantoin. In both instances the dosage of nitrofurantoin was relatively low 150 mg daily and the medication had not lasted more than 30 days. As her renal function was normal, it seems unlikely that there had been any marked accumulation of the drug as an explanation of the complication. Moreover the beginning of the remission after three days treatment with prednisone in addition to the withdrawal of nitrofurantoin is a pattern of recovery that holds good for most cases of agranulocytosis (3) and would favour an immunological reaction rather than toxic effect on the bone marrow as the pathogenetic mechanism of the action of the drug (3).

Our case shows convincingly that we must include nitrofurantoin in the list of potential causes of agranulocytosis.

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Congress Announcements

The 6th International Diagnostic Course on pulmonary diagnosis (lungs, mediastinum, pleura) will be held in Davos, Switzerland, April 4-10, 1974. Sponsored by the European Association of Radiology.

Information. ID&D P.O. Box 159 CH-8033 Zürich, Switzerland.

An IAEA Symposium on dynamic studies with radiolabels in clinical medicine and research will be held in Knoxville Tennessee, USA, July 15-19 1974.

Organizers. International Atomic Energy Agency Kärntner Ring 11-13 P.O. Box 590 A-1011 Vienna, Austria.

Scientific Secretaries. R. A. Dudley and E. H. Bleicher Medical Applications Section.

Abstracts must be submitted before Feb. 8 1974 through the national authorities for atomic energy matters.

The Third International Congress of Parasitology will be held in the Kongress-Zentrum, Messgelände Munich, West Germany Aug. 25-31 1974.

President G. Piekarski, Institute for Medical Parasitology of the University D-5300 Bonn Venusberg 1 West Germany

Secretary General G. Lämmler Institute for Parasitology of the University R. Buchheim-Str. 4 D-6300 Giessen, West Germany

The XII International Congress of Internal Medicine will be held in Tel Aviv Israel, Sept. 8-13 1974.

Sponsor International Society of Internal Medicine.

Organizer Israel Society of Internal Medicine, *Secretary General of ISIM* Dr Ph. C. Frel, Department of Immunology and Allergy Hôpital Neufel, CH 1011 Lausanne, Switzerland.

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The 4th Congress of the International F of Manual Medicine will be held in Prague Czechoslovakia, Oct. 9-12, 1974.

Congress languages English, French, German, Russian and Czech or Slovak (with simultaneous translation).

Secretariat 4th Congress of the International Federation of Manual Medicine Sokolská 31 120 26 Praha, Czechoslovakia.

The XI International Cancer Congress will be held in Florence, Italy Oct. 20-26, 1974. During first two days ten conferences will be held in different towns. The topics will be: 1) Cell biology 2) Chemical carcinogenesis, 3) Molecular biology of viral oncogenesis, 4) Tumour immunology 5) Environmental factors in cancer induction. Appraisal of epidemiological evidence, 6) Detection of preclinical cancer 7) Biological concepts in surgical management of cancer 8) Advances in cancer chemotherapy 9) Progress in radiobiology and radiotherapy 10) Cancer campaigns. The following three days will be devoted to symposia and proffered papers. All sections will be held in Florence. Advanced courses on clinical oncology will be given in Florence on Oct. 26.

Official languages. English, French, Italian.

Registration fees (US \$): members 150, associate members 60 advanced courses on clinical oncology 10. Payment must be made to Canc. Congress, c.c. 17129/00 Cassa di Risparmio V. Bufalini 4 Firenze, Italy.

Proffered papers on kindred subjects will be grouped together and presented in the form of workshops. Deadline for summaries of papers Feb. 1 1974 (not exceeding 250 words, in one of the official languages, clearly typed using double spacing for reproduction by photo-offset process). Each member may present only one paper.

Information. General Secretariat, XI International Cancer Congress, Via G. Venezian 1 20133 Milano Italy.

